Effects of biostimulants on soil microbiota, plant development, crop productivity and fruit quality of protected strawberries

Mémoire

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Résumé

La culture de la fraise (*Fragaria x ananassa* Duch.), l'une des plus importantes productions horticoles au Canada, fait face à des défis importants pouvant affecter la productivité et la qualité des fruits. Par conséquent, cette étude se concentre sur l'utilisation des biostimulants les plus prometteurs pour les fraises pouvant améliorer le microbiote du sol, le développement, la productivité et la qualité des fraises produites sous abris.

Deux expériences en blocs aléatoires complets ont été réalisées en serre et sous grands tunnels. Dans l'essai en serre, nous avons étudié l'effet de 14 traitements sous gestion conventionnelle (7 traitements) et biologique (7 traitements). Pour le système de culture conventionnelle, les traitements consistaient en: 1- Témoin (sans biostimulant), 2- Extrait d'algue, 3- *Trichoderma harzianum* souche T22, 4- *Rhizoglomus irregulare*, 5- Combinaison d'*Azospirillum brasilense, Gluconacetobacter diazotrophicus* et *Bacillus amyloliquefaciens*, 6- Mélange des traitements 4 et 5, et 7- Formulation à base d'acide citrique. Pour le système de culture biologique, les traitements biostimulants étaient: 8- Témoin (sans biostimulant), 9- Extrait d'algue, 10- *Rhizoglomus irregulare*, 11- Combinaison d'*Azospirillum brasilense, Gluconacetobacter diazotrophicus* et *Bacillus amyloliquefaciens*, 12- Mélange des traitements 10 et 11, 13- Mélange des traitements 10 et 11 à faible fertilisation, et 14- Formulation à base d'acide citrique, dans une conception de blocs aléatoires complets avec cinq répétitions. D'autre part, dans un essai sous grands tunnels, nous avons étudié six traitements 1- Témoin (sans biostimulant), 2- *Rhizoglomus irregulare*, 3- Combinaison d'*Azospirillum brasilense, Gluconacetobacter diazotrophicus* et *Bacillus amyloliquefaciens*, 4- Mélange des traitements 2 et 3, 5- Formulation à base d'acide citrique et 6- Formulation à base d'acide citrique et lactique à l'intérieur d'un dispositif expérimental en blocs aléatoires complets de quatre répétitions.

Nos résultats ont montré que les paramètres d'activité du sol étaient plus élevés sous une gestion de culture biologique, bien que les traitements de biostimulants n'ont pas augmenté l'activité microbienne du sol par rapport à leurs contrôles respectifs, à l'exception de la combinaison de mycorhizes et de bactéries pour des plantes cultivées conventionnellement sous grands tunnels. Pour les deux expériences, les biostimulants n'ont pas influencé significativement la performance photosynthétique des feuilles. Cependant, les biostimulants ont eu un impact sur le développement des plantes et certains paramètres de croissance. En serre, les mycorhizes sous régie biologique et le traitement de mycorhizes et de bactéries sous régie conventionnelle ont diminué le

nombre de tiges florifères par rapport aux plantes témoins. En revanche, tous les biostimulants ont augmenté la croissance des plantes cultivées sous grands tunnels. En serre et sous régie conventionnelle, le rendement des plantes traitées avec l'acide citrique a été supérieur à celui des plantes témoins, tandis que l'acide citrique et une combinaison de mycorhizes et de bactéries sous régie biologique a augmenté le rendement. Sous grands tunnels, aucun effet significatif sur le rendement n'a été observé. Le traitement de mycorhizes et de bactéries a augmenté la teneur des fruits en ºBrix, en polyphénols et en anthocyanines des plantes cultivées en serre et sous régie biologique, tandis que *Trichoderma* a augmenté la teneur en polyphénols et en anthocyanines des fruits sous régie conventionnelle. Aucun effet des biostimulants sur le contenu des fruits en ºBrix et polyphénols n'a été observé sous grands tunnels, tandis que tous les biostimulants ont augmenté la teneur en anthocyanines des fruits.

D'après cette étude, nous pouvons conclure que certains biostimulants ont montré des effets bénéfiques, permettant ainsi d’améliorer la performance agronomique de la fraise en termes de croissance, de rendement et de la qualité des fruits de plantes cultivées sous abris. La variabilité observée entre les deux systèmes de production confirme l'importance de la validation de ces résultats sous différentes conditions de croissance et saisons de production.

Abstract

The strawberry (*Fragaria x ananassa* Duch.) is one of the most important horticultural crops in Canada. However, several challenges limit the productivity and quality of this crop. Therefore, this study focused on using the most promising biostimulants that can improve soil microbiota, plant development, crop productivity, and berry quality in the greenhouse and high tunnels.

In order to study different biostimulants treatments, a greenhouse and high tunnel experiments were carried in a complete randomized block design with five or four replicates. For the greenhouse trial, we studied the effect of 14 treatments under conventional (7 treatments) and organic (7 treatments) growing management. Studied treatments for the conventional growing system consisted of 1- Control (without biostimulant), 2- Seaweed extract, 3- *Trichoderma harzianum* strain T22, 4- *Rhizoglomus irregulare*, 5- Combination of *Azospirillum brasilense, Gluconacetobacter diazotrophicus* and *Bacillus amyloliquefaciens*, 6- Mixture of treatments 4 and 5, and 7- Citric acid-based formulation. For the organic growing system, the biostimulant treatments were: 8- Control (without biostimulant), 9- Seaweed extract, 10- *Rhizoglomus irregulare*, 11- Combination of *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus* and Bacillus amyloliquefaciens, 12- Mixture of treatments 10 and 11, 13- Mixture of treatments 10 and 11 with low fertilization, and 14- Citric acid-based. For the high tunnel experiment, six treatments were compared: 1- Control (without biostimulant), 2- *Rhizoglomus irregulare,* 3- Combination of *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus* and *Bacillus amyloliquefaciens*, 4- Mixture of treatments 2 and 3, 5- Citric acid-based formulation, and 6- Citric and lactic acid-based formulation.

Our results showed that soil activity parameters were higher under organic crop management compared with the conventional one, although biostimulant treatments did not increase soil microbial activity compared with their respective control, except for the combination of mycorrhiza and bacteria of high tunnel conventionally grown plants. For both experiments, biostimulants did not influence significantly leaf photosynthetic performance. However, biostimulants did impact plant development and some growth parameters. Compared with control plants, our results showed that the number of flowering stalks decreased for greenhouse organically grown plants treated with mycorrhiza and for conventionally grown plants treated with the combination of mycorrhiza and bacteria. On the other hand, all biostimulants increased the growth of plants grown

under the high tunnels. Concerning yield parameters, conventionally grown plants treated with citric acid produced higher total and marketable yield compared with control plants, while the marketable yield of organically grown plants was higher in the plants treated with citric acid and the combination of mycorrhiza and bacteria. In contrast to the greenhouse experiment, no yield effect was observed for high tunnel plants. In terms of berry quality, *Trichoderma* increased the polyphenol and anthocyanin content of conventionally grown berries, while a combination of mycorrhiza and bacteria increased the ºBrix, polyphenol and anthocyanin content of organically grown plants compared with control. No effect of biostimulants on ºBrix and polyphenols were observed for high tunnel plants compared with control, while all biostimulants increased berry anthocyanin content.

From our study, we can conclude that some biostimulants may improve strawberry performance in terms of growth, yield, and fruit quality. The lack of a significant difference between biostimulant treatments, due to large variability, confirms the importance of validating these results under different growing conditions and production seasons.

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Introduction

In 2018, fruit production accounted for 20% of overall edible horticulture cash receipts with a farm gate value of 123,273 million dollars in Canada, which increased by 6.1% from 2017 to 2018 [1]. Among fruits, berries are one of the small health improving fruits among consumers worldwide by having beneficial effects on dietary and reducing the cardiovascular diseases [2, 3]. Strawberries are one of the most consumed and cultivated horticultural crops in the world [1, 4]. For growers, the important aspects of strawberry production are achieving high yield of high quality. However, increasing productivity and quality of strawberries face some major issues. The most important challenges are pests, soil diseases and for organic crops, the soil nutrient balance and nutrient availability.

Conventional methods such as application of pesticides, soil fumigants, and chemical fertilizers are largely used to increase the productivity of strawberries. However, it has been shown that conventionally practices are not sustainable and have several negative effects on the ecosystem [5]. Besides, there are several problems with fumigants. The most important problem is its negative effect on the environment as well as workers who are dealing with this practice [6]. This happens because the widely used pest controlling method is not selective, and it affects beneficial microorganisms as well as targeted pathogens [7]. Besides the negative effects of pesticides on the environments, they are expensive in terms of profitability and inefficient in several cases due to pesticide resistance or their misuses.

On the other hand, demand for organic food in Canada increased by 4-fold during the last decade, with 39% represented by fruits and vegetables [8]. For strawberries, the plus value of organic strawberries in 2017 was in average 64% higher than the conventional price with a plus value reaching 308% in November [9]. Furthermore, strawberry was reported as one of the fruits with the highest level of pesticide residue, which might have a significant impact of the health of the population, particularly young children that love strawberries. Therefore, in order to achieve sustainable yield of high-quality fruits, alternative approaches are required to reduce chemical inputs, without reducing crop productivity.

Biostimulants, are considered exogenously applied as substances to plants to improve nutrition efficiency and quality attributes regardless of its nutrient content [10]. Besides, they can improve plant resilience to abiotic stresses (e.g. salinity, water stress) and biotic

stresses (e.g. root diseases). The interest for biostimulants is expanding quickly worldwide as they constitute promising alternatives to unsustainable approaches. In fact, the global biostimulant market was forecasted to reach 2.241 million US $ in 2018, with an annual growth of 12.5% [11]. However, little is now about the benefit of adding biostimulants under organic farming as organic amendments already constitute a source of beneficial fungi and bacteria (PGPR), humic acids as well as organic (e.g. amino acids, chitin) and inorganic components (e.g. Si).

Several reviews [10-15] and articles on biostimulants have been published in recent years. However, most of the studies were focused on the improvement of plant resilience [16-18], antifungal effects [19], and plant development [20] of conventional growing crops. Based on recent studies, we have selected the most promising biostimulants for strawberries that can improve plant development, crop productivity and berry quality under conventional and organic growing conditions.

Hypotheses and Objectives

This study proposed the use of different biostimulants as a sustainable alternative to improve the yield and quality of strawberry berries. The hypotheses that we have verified in this study are as follows:

1) In organic and conventional growing systems, biostimulants (seaweed extract, *Trichoderma* spp., mycorrhiza, nitrogen-fixing endosymbiosis bacteria, endosymbiotic nitrogen scavengers, phosphates and potassium solubilizing bacteria as well as organic acids) increase plant development and crop productivity by improving plant nutrient uptake.

2) Biostimulants (organic acids, *Trichoderma* spp., seaweed extract, mycorrhiza, endosymbiotic nitrogen scavengers, nitrogen-fixing endosymbiosis bacteria as well as phosphates and potassium solubilizing bacteria) improve the berry quality in terms of appearance, taste and nutritive value in both conventional and organic growing management.

The main objective of this study was to identify the benefits of the selected biostimulants in terms of plant development, crop productivity and fruit quality of conventional and organic berries grown under protected environment.

The specific objectives were

1) to compare the agronomic performance of plants grown with and without biostimulants;
2) to study the effects of biostimulants on berry quality in terms of appearance, taste and the nutritive value;

3) to characterize the impact of some biostimulants on the indigenous soil microbiota; and
4) to validate the more promising results observed under greenhouse conditions to high tunnels.

1 LITERATURE REVIEW

According to FAO, 9,223,815 tons of strawberries cultivated on 395,844 ha (233,017 kg/ha) were produced in the world in 2017. The major producer of this crop is China with 2,860,008 tons of strawberries and followed by USA with 1,234,134 tons Mexico 658,436, Turkey 400,167, and Egypt 318,950. [21]. In the following year, Canadians produced 29,809 tons of strawberries on 3,904 ha with a farm gate value of 123,379 million Canadian dollars [1]. Among the Canadian producing provinces, Quebec is the leading province in terms of strawberry production by producing 57% of the total national strawberries in 2018 [1].

1.1 STRAWBERRY (*FRAGARIA* X *ANANASSA* DUCH.)

Strawberry, an herbaceous, dicotyledonous, and perennial plant, is a member of the *Rosaceae* family. This plant results from a cross between *F. virginiana* and *F. chiloensis* [22]. The anatomical structures of the strawberry plant are a central stem (crown), a root system, leaves, runners or stolon, inflorescence (stems with primary flowers), and axillary crowns (see Figure 1.1) [23]. Strawberry plants form a rosette and leaves are arranged in a spiral shape. This crop grows in two phases: vegetative and reproductive. In the vegetative phase, which starts from germination until the reproductive phase, plants increase the size and produce vegetative structures. The crop produces both runners and flowering fruit stalks in the second phase. The 50-90% of the strawberry roots are in the first 10-15 cm of the soil [22].

Strawberry plant shows a high level of adaptation in different environments and growing conditions [24]; as a result, it can grow on all cultivated areas in the world from the northern to tropical lands. Generally, this crop needs sandy loam soil with pH of 5.5-7.0, good drainage conditions, moderate irrigation and fertilization, sunny locations, and temperature of 15-30°C [22]. According to Helman and Travis [25], temperature between 35 and 40°C is the critical point which inhibits strawberry growth.

The commercial strawberry plants are multiplied from plant division, seeds, and runners [26, 27]. Runners are used to produce a plug or tray plant strawberries which are an alternative method of strawberry propagation. Tray plants are mostly used for strawberry production in either a high tunnel or a greenhouse [28]. These plants have several benefits for strawberry production. Firstly, tray plants minimize the use of pesticide and reduce the

soil-borne diseases such as verticillium wilt (*Verticillium* spp.) and Phytophthora root rot (*Phytophthora* spp.) [29, 30]. Secondly, producers can conveniently transplant the plug plants either manually or mechanically. Thirdly, plug plants can establish well under overhead-sprinkling water applications, and this can improve water management after planting [29, 31]. These advantages motivated us to use plug plants as the type of strawberry in our investigation.

Beside strawberries soft texture, and sweetness, this crop is an essential source of fibres, vitamins like vitamin C, carotenoids [32], natural antioxidants [33], aroma [34] and essential nutrients like potassium, phosphorus, calcium, and iron [32, 35]. There is a wide variety of volatile aroma (more than 360 volatiles) in strawberries. Precisely, fruit aroma consists of a mixture of terpene alcohols, furanone, esters, lactones, and sulfur compounds [34]. Among the important flavorful components of strawberries, furaneol [2,5-dimethyl-4-hydroxy- 3(2H)-furanone] and mesifurane [2,5-dimethyl-4-methoxy- 3(2H)-furanone] are two major aroma volatiles [36].

Strawberries are also rich in phenolic compounds such as polyphenols and anthocyanins [32, 37-39]. Da Silva [40] reported 25 different anthocyanin pigments in several cultivars of strawberries. In fact, anthocyanins are the primary source for the red color of the berry. Genetic background, environmental conditions, growth, ripening stage and storage temperature are essential factors which affect the content of phenolic compounds in the strawberry fruits [32, 41]. In this study, we have investigated the effect of several biostimulants on the phenolic compounds and anthocyanins.

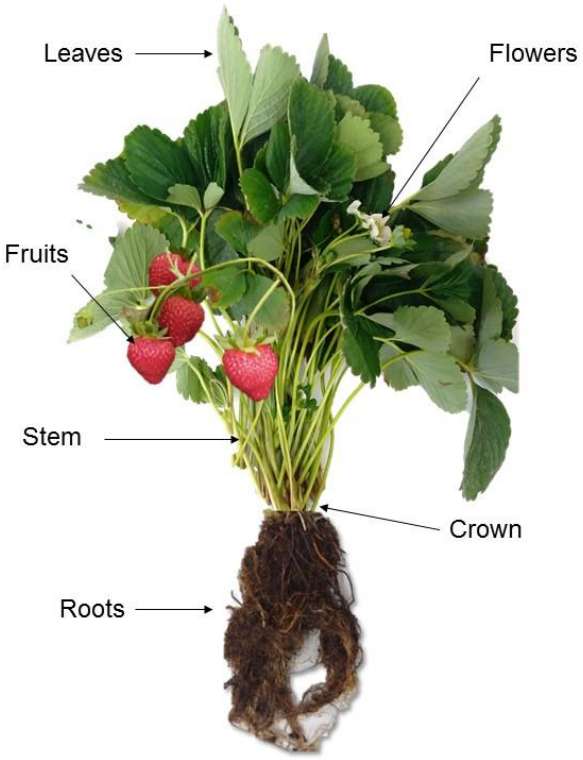

Figure 1.1 Anatomical structures of the strawberry plant.

1.1.1 Types of cultivars

Strawberry cultivars are classified into three main subgroups based on photoperiod and temperature responses on flowering behaviors: long-day, short-day or June-bearing and day-neutral. In most of the research, short-day, and day-neutral strawberries are the main types of cultivars used for the commercial production [22]. Short-day varieties need a daylength lower than 11-16 h and temperature of 15°C for its flower buds' initiation [42, 43] [22]. Researchers consider the cultivars as day-neutral if the effect of photoperiod on the flower initiation is not essential or absent [44]. This means that regardless of the photoperiod, plants will continue to be flowering [22].

In Canada, growers cultivate several varieties such as Monterey a day-neutral variety [45]. We choose this cultivar because of its good yield and the quality of its fruits. Monterey is resistant to two spot mites (*Tetranychus urticae*) and strawberry viruses. Additionally, this cultivar has more abundant fruits, better flavor, broader leaves with thicker and higher petiole length than other varieties. Monterey is moderately resistant to verticillium wilt

(*Verticillium dahliae*) and anthracnose crown rot (*Colletotrichum acutatum*), and moderately sensitive to powdery mildew (*Podosphaera aphanis*), leaf spot (*Ranularia tulasnei*) and Phytophthora crown rot (*Phytophthora cactorum*)., which are important strawberry plant diseases [46].

1.2 EFFECT OF PRODUCTION SYSTEMS

Genotype, temperature, and the planting date affect strawberry production in terms of quality and marketable yield. Generally, growers are producing strawberries in the field, high tunnels, and greenhouses on raised beds, hill rows, matted row system (MRS), and soilless cultures and hydroponics. Hill rows is often used for year-round production [47]. In this system, strawberries are transplanted in early fall for winter and spring season in Baton Rouge, Louisiana [48].There are two kinds of hill rows: single row bed (used in Pacific Northwest) and double row bed (used in Florida and California). Strawberries are usually transplanted in the hills with black plastic covers to increase the vegetative growth and minimize the soil-borne disease (use of preplant fumigants) and weeds. Under conventional farming, plants are fertilized by the drip irrigation system, which is under the plastic mulch [47, 48]. Several factors affect the performance of this system, including the health and size of the plant and the environmental conditions such as temperature [48, 49].

Matted row system is the widely used production system in northern regions due to the convenience of the method and lower cost [47, 50]. Strawberries obtained are generally of better quality and size when narrow beds are used and competition between plants is lower. Besides the low cost, several studies reported the advantages of the matted row systems. For example, Black et al. [51] found that plastic mulch and soil fumigants are not needed under the matted row system. However, the main disadvantage of this system is the poor harvest efficiency, fertilizer waste, and fruit rot [50].

According to Savvas et al. [52], soilless culture refers to "*any method of growing plants without the use of native soil as a rooting medium, in which the inorganic nutrients absorbed by the roots supplied via the irrigation water*". In this hydroponic system, plants are grown in water [53] or in inert substrate [52]. There are several kinds of substrate materials, such as minerals, organic, and artificial growing media. Plants are transplanted into one of these materials or their mixture in the gutters, slabs, bags or containers [54].

Jafarnia et al. [55] reported that the most common substrates for strawberries are peat, perlite, coconut fibre, rockwool, pine bark and tuff.

Soilless production systems have several advantages. First, the control of soil-borne pathogens is easier, which makes these systems to be a safe alternative to chemical products [54, 56]. Secondly, we can better manage the water and nutrients in soilless production due to optimal physicochemical properties of the growing media [53]. Jafarnia et al. [55] showed an increase in greenhouse strawberries yield by using the soilless growing system. In another study, Cecatto [57] reported that the quality of the strawberry fruits grown in soilless growing media was better than soil cultivation in terms of total soluble solids, size, and acidity of the fruits. According to Martínez et al. [58], soilless culture and growing media had significant effects on the population of the microorganisms in the rhizosphere. In another study, Martínez et al. [59] reported that strawberry yield (size and weight of the fruit) and fruit quality (firmness, polyphenol, and anthocyanin content) of strawberries grown in coir fibre were higher than plants grown in the soil. Many other studies support the beneficial effects of soilless culture for the production, growth and development of the strawberry plants [60-62]. All the discussed studies, however, did not investigate the effects of growing media under organic and conventional condition, which motivate us to put hands-on for this investigation.

More recently, most of the researchers have recommended protected cultivation such as greenhouse and high tunnel for year-round strawberry production. The most important effect of protected cultivation is minimizing the risks of pest pressures and environmental unfavorable condition [63], more often observed by growers due to climate changes. There are several pros and cons of greenhouse and high tunnel strawberry cultivation. In the greenhouse, we can handle water, light, CO_2, and temperature easily compared to open field production. Furthermore, in some growing areas, the cooling system inside the greenhouse allows having better production during the hot summer season as strawberries like a relatively low night temperature. However, these high tech greenhouses have a higher cost in terms of capital investment, carbon dioxide emission, energy and high operational costs (needed more workers) [64].

High tunnels are the most used protected cultivation system compared to the greenhouse from an economic perspective. In North America, growers generally use multi- or single bay high tunnels for commercial small fruit production, such as strawberries [65]. As

discussed before, the gutter system and raised beds are preferred production systems in high tunnels. Although high tunnels do not have permanent automated heating and ventilation systems as greenhouses, they provide warmer environment compared to field production (ref). Besides, high tunnels extend the growing season and protect the crops against pests, wind, rain and hail [66]. For example, Kadir et al. [67] reported that high tunnels protect strawberries from winter damage, and this method helps for high quality and quantity production. In terms of pest management, Xiao et al. [68] reported several advantages of high tunnel cultivation for strawberries. Indeed, they showed that the incidence of the Botrytis fruit rot (*Botrytis cinerea*) and powdery mildew (*Podosphaera aphanis* f. sp. *fragariae*) was lower in the high tunnel growing system compared to the field because of a short period of leaf wetness. Additionally, strawberries (cv Sweet Charlie) yield in the early season was 8-13% higher in the high tunnel compared with the field.

1.3 EFFECT OF MINERAL NUTRITION ON STRAWBERRY PLANTS

For higher productivity and quality, strawberry plants require an excellent nutrition condition. Mineral nutrients, including macro- and microelements, affect plants due to their influence on plant growth, development, yield, and quality of the fruits. However, finding the critical and optimum levels of these elements for increasing the yield and quality is not a trivial task [69]. In this subsection, we will briefly cover some of the essential minerals which will be introductory for chapters 2 and 3 to understand the general goal of this study.

After carbon, nitrogen (N) is a significant element in the plant nucleic acids, amino acids, chlorophyll, and other compounds [70, 71]. This element is available in different kinds as a fertilizer such as ammonium (NH_4^+) and nitrate (NO_3^-) ions [71]. Nitrogen plays a vital role in the growth, development, yield (fruit firmness and size), resistance to pests and diseases, and quality of all plants including strawberry plants [62, 72, 73]. According to Tabatabaei et al. [74], the ratio of NH_4^+: NO_3^- were affected the total N absorption by strawberry plants. They reported that a NH_4^+:NO_3^- ratio of 1:3 was the optimal ratio to increase plant growth, yield, and quality of strawberry fruits (cv. Camarosa and Selva) in a mixture of perlite and vermiculite (1:1). In addition, the pH of the rhizosphere and the source of N-fertilizer can affect nutrient uptake and nutrient solubility [75].

Besides nitrogen, phosphorus is the other important element in the plant in terms of macromolecular structure and nutrition [75, 76]. It is available for the plant as hydrogen

phosphate (HPO_4^{2-}) and dihydrogen phosphate ($H_2PO_4^-$) ions [71]. Phosphorus plays a critical role in growth, performance, and development, especially for root development [76, 77]. According to Choi et al. [78], the concentration of the phosphorus is important for better above-ground plant biomass.

Potassium is another element found in large quantities in the leaf and fruit tissues. The plant absorbs potassium in the form of potassium ions (K^+) [79]. This element is responsible for stomatal opening and closing, translocation of sugar, starch formation, activation of enzymes, cell osmoregulation and consequently plant growth [80]. Bibi et al. [81] reported the positive effects of the potassium on the strawberry plant. They showed that potassium (60 g/m^2) increased plant growth and yield parameters of strawberries. Besides, potassium makes huge variations in physiological, morphological, and agronomical characteristics of the strawberry plant. Therefore, this element can increase productivity and quality.

Other elements such as calcium, magnesium, and sulfur are also structural elements in the plant tissues. For example, calcium is involved in the cell membrane and cell physiology. It also has a vital role in the fungal and bacterial infection tolerance [82]. On the other hand, magnesium and sulfur are the structural components of the chlorophyll molecule, and they are involved in photosynthesis [79, 80].

Although not considered as an essential element, silicon (Si) is now recognized as a beneficial nutrient [83, 84]. For example, several researchers have shown that silicon is beneficial against several biotic and abiotic stresses [85-89]. This element is available in different types of fertilizers such as silicate salt or liquid form (potassium silicate) and can be applied in the irrigation water or soil [85, 86, 88, 90, 91]. Si is also considered as a biostimulant and will be covered in the following sections.

1.4 CONVENTIONAL VS ORGANIC

Today, most of the strawberry producers are using a conventional system using pesticides and soil fumigant methods. This system is mostly used to control pests and to increase the yield. However, today, we know that these practices are not sustainable and have several negative effects on the ecosystem. They are expensive in terms of economy and inefficient in some cases [9]. Although its beneficial health components [92], strawberry

fruit is recognized as one of the fruits having a high level of pesticide residues [93]. This might have a significant impact on the health of the population, particularly the children. As a sustainable alternative to conventional farming, researchers investigated organic agriculture methods, while producing high yield of high quality [9].

Today's society puts more attention to organic horticulture. According to International Foundation for Organic Agriculture Movements [8], organic farming is defined as a system that aims to maintain the health of soils, ecosystems, and humans. Organic systems do not allow the use of synthetic pesticides and fertilizers, growth regulators, antibiotics, and genetically modified products [94]. The value of the organic food market has grown by 5.8 times over the last 15 years, reaching 97 billion USD in 2017. Organic strawberry represented around 8383 ha in the world [8]. In Canada, demand for organic food increased by four times during the last decade [8]. In North America, about 2588 ha area is under organic strawberries [8].

One of the defining aspects of organic horticulture is fertilization since only certain amendments are permitted in a perspective of maintaining fertility and soil health and limiting negative effects on the environment. Manure and compost amendments which are rich in organic matter, play an essential role to stimulate the biological activity of soils [94, 95]. As a result, the enzymatic activity and microbial biomass are high in the organic systems.

Tucker [96] used organic manure in strawberry production. He reported that the vegetative growth increased by using organic manure compared to the chemical fertilizers. Then, in 1936, Wallace [97] reported the high yield of the strawberries with organic manures. Abou-El-Hamd et al. [98] showed that the total sugars and anthocyanins of the strawberry fruits increased in the plants treated by organic manure compared with the chemical fertilizers. According to Ceglie et al. [99], strawberries grown in the conventional system have a high diameter, firmness, and chroma value compared to organically produced fruits. However, their results showed that the quality parameters of the berries (Vitamin C, sugar content, and acidity) increased in the organic system. Furthermore, Reganold et al. [100] reported the beneficial effects of organic farming on the quality of strawberry berries, health of the soil, and plant resilience to stresses via increasing the microbial activity.

Beside advantages that affect plant and environment, disadvantages are associated to organic farming. First, the cost of the process may be higher due to labor-intensive management. Secondly, the plants often face more diseases, weeds, and pests because of the absence of fumigants, herbicides, and pesticides in the organic system [95]. Consequently, the produced strawberries in the organic system could suffer from lower yield compared to conventional ones, although some organic growers may achieve higher yield than conventional crops. This yield decrease is on average 20% for horticultural crops [101]. One of the main sources of this decline is the soil disease, which limits the productivity in Canada like in Europe and the United States [73, 102]. To control soil diseases under conventional farming, growers mostly use fumigants. In addition to disease, nutrients are less available in the organic system due to the absence of regular or well-balanced fertilizer inputs [103] or/and the mismatch between the nutrient mineralization rate and plant nutrient needs. Therefore, these challenges motivate researchers to look for alternative methods under organic farming.

1.5 BIOSTIMULANTS: AN ALTERNATIVE FREE- CHEMICAL METHOD

As discussed above, depending on the plants, organic agriculture suffers from lower yield (5-32%) compared to the conventional agriculture [101, 104, 105]. Nowadays, researchers are using biostimulants in organic farming to partly solve the issue of less productive crops [14] and to improve plant resilience to biotic and abiotic stresses. The results of recent studies showed that biostimulants are also an essential and promising alternative to reduce the use of pesticides [106, 107]. Additionally, biostimulants are an innovative supplement for conventional farming, which results in better crop nutrition and protection. In the global market, European countries are the largest users of biostimulants with 40% of 2.19 billion USD in 2017, and the global market for biostimulants projected to reach 3.29 billion USD by 2021 [108]. The concept of biostimulants and their categories will be discussed in the following sections.

1.5.1 What is biostimulants?

The origin of the term “biostimulant” backs to 1950s. “Biogenic-stimulators” is proposed by Filatov [109] for the first time and was defined as: “*biological material which obtained from various living tissues (organisms, animal, human, and plant). These stimulators can be affected by metabolic and energetic processes and can stimulate the life reactions on the organisms*”. Then, several studies applied the idea of biogenic stimulants to plants,

with the goal of improving plant enzyme activity with dibasic characteristics contained (like) organic acids [110]. In 1994, Herve [111] proposed the first approach for applying biostimulants on plants. According to Herve [111], the new concept of "bio-rational products" should have a systemic strategy based on chemical synthesis, biochemistry, and biotechnology to overcome the limitations in crop physiology, and more generally in plant agriculture. Herve [111], also, suggested some rules, like a low dose of usage, for "environmentally friendly" and reproducibility.

The European Biostimulant Industry Council (EBIC) describes biostimulants as "*organic or natural material products obtained from bioactive materials and/or microorganisms that can boost several molecular and physiological processes.*" According to EBIC, the following issues must be covered in the definition of biostimulants [112]:

I) recognition of the systematic effects of biostimulant components;
II) specifying the indirect impact of biostimulants on plant growth and direct effect on the soil microflora;
III) clarifying the positive effects of biostimulants on yield and improvement of the quality by increasing the crop development.

Consequently, biostimulants are different from conventional crop inputs in two respects. First, biostimulants affect different mechanisms of plants regardless of their nutrient content compared to fertilizers. Secondly, biostimulants could have an impact on the vigor of a plant, without having a direct effect against pests and disease. Therefore, they differ from crop protection products.

For better clarification of the difference between biocontrol substances and agents with biostimulants, there are other definitions of biostimulants in the current literature. According to the *Biostimulant Coalition* in North America biostimulants are defined as "*substances, including microorganisms, that are applied to plant, seed, soil or other growing media that may enhance the plant's ability to assimilate applied nutrients or provide benefits to plant development. Biostimulants are not nutrients and therefore may not make any nutrient claims or guarantees*" [113]. As an alternative definition, Du Jardin [10] defined biostimulants as "*any substance or microorganism applied to plants to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content*." Additionally, Yakhin et al. [107] suggested a similar definition of biostimulants as "*a formulated product of biological origin that improves plant productivity*

as a consequence of the novel or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds."

Upon these definitions, we can summarize the overall roles of biostimulants as follows:

I) increasing the performance of the plant (e.g. photosynthesis and plant resilience to stresses);
II) improving water use efficiency and increasing the nutrient uptake from the soil (e.g. N, P, Fe);
III) helping for soil structure and increasing the tolerance and recovery from abiotic stresses such as salt, water, heat, and heavy metals;
IV) enhancing the plant growth;
V) promoting productivity and quality, which was one of the goals of this study [11, 13, 20, 107, 113, 114].

As a result of these advantages, we can reduce the plant's chemical inputs, including fertilizers, the production cost, and the environmental burden by using biostimulants — these lead researchers for applying different biostimulants in different plant species in recent years [15, 115].

1.5.2 Categories of biostimulants

Researchers categorized biostimulants into different groups. In one of the first categorizations, Filatov [116] proposed four groups of the biogenic stimulants. Then, Karnok [117] categorized 15 biostimulants in a list of 59 substances and components. Next, in 2004, Ikrina and Kolbin [118] proposed nine natural raw materials for classifying the plant biostimulants. Later, Kauffman et al. [119] categorized the organic biostimulants in three groups: seaweed extracts, humic substances, and products containing amino acids. In the next year, Basak [120] classified biostimulants according to their mode of activity and the origin of their active ingredient. Recently, Du Jardin [10] grouped the biostimulants in seven categories: 1) humic and fulvic acids, 2) protein hydrolysates and other N-containing compounds, 3) seaweed extracts and botanicals , 4) chitosan and other biopolymers, 5) inorganic compounds, 6) beneficial fungi, and 7) beneficial bacteria. At the same year, La Torre et al. [121] proposed a similar classification of the biostimulants. To sum up, researchers classified biostimulants based on their functions, applications, the active ingredient, and their type of activity [107].

In the following subsections, we will first briefly cover the categories of biostimulants defined by Du Jardin [10] and Toscano et al. [122] that are related to our study. Consequently, four categories of biostimulants will be discussed: humic substances (HS), hydrolyzed proteins and amino acid containing products (AACP), hormone-containing products such as seaweed extracts (SE), and microorganisms. Additionally, we have studied a new category of biostimulants, called organic acids, on the strawberry plant under both organic and conventional farming.

1.5.2.1 Humic substances (HS)

Humic substances (HS) are one of the most abundant biostimulants on Earth, which are derived from the metabolic activities of the soil microbes [10, 123, 124]. Specifically, HS result from the decomposition of the microbial, animals, and plant's residues. Generally, HS are classified into humic acids, humins, and fulvic acids based on their solubility property and molecular weights. Except for controversial cases, [123, 125], it was reported that HS of low molecular weights resulted in better impacts on crops [126, 127].

Several studies showed that HS increased the number of fruits, flowers, and the quality of the fruits of marigold, pepper, strawberries, and tomatoes grown under greenhouse condition [128, 129]. Additionally, HS can change the morphology of plant roots, increased ATPase activity, and improved the action of the nitrate assimilation enzymes [130]. Furthermore Halpern et al. [131] showed that HS promoted the soil structure and the nutrients (N, P, Mn, Cu, Zn, and Fe) plant uptake from the soil for barley. In the same year, Du Jardin [10] reviewed several studies. He proposed that the optimal root interactions between plants, organic matter, and microbes are required to increase the crop yield by humic substances. HS can be applied into the irrigation water, or directly in the soil [131]. It is noted that we should take care of several factors like the type of plant and environmental conditions for optimal results of HS.

1.5.2.2 Hydrolysed proteins and amino acid containing products (AACP)

As the second categories of biostimulants, amino acids and protein hydrolysates (PHs) can increase plant growth in many cases [11, 131, 132]. These compounds are formed by thermochemical, enzymatic hydrolysis of the dedicated biomass crops [133], plant sources (crop residues), and animal wastes such as collagen and epithelial tissues [131, 132, 134]. Amino acids and protein hydrolysates showed several effects on plants and the soil. For example, Shehata et al. [135] reported that the application of amino acids (AA) could

increase biomass production. In several studies, researchers proposed AA for protecting plants from biotic and abiotic stresses [136-140]. Ardebili et al. [141] reported the enhance of antioxidation levels of *Aloe vera* L. by using AA. As an exogenous application, AA can improve the uptake process of nutrient in plant roots and leaves [131]. They can also stimulate root branching by inhibiting primary root growth [142]. Additionally, García-Martínez et al. [143] proposed the application of AA to improve the physical and chemical characteristics of the soil via improving microbial activity. Specifically, they showed that organic matter could decompose rapidly by the increase of soil bioactivity, resulting in transforming of organic nutrients into plant-available mineral forms.

1.5.2.3 Seaweed extracts (SE)

Seaweed extracts (SE) are organic and mineral components which contain different types of hormones e.g. auxins, cytokinin, abscisic acid as well as amino acids [144, 145]. Generally, they are applied in two ways: at the root level or as foliar applications [77]. SE are heterogeneous compounds which characterized according to their parental material, the extraction solution pH, and 1H-NMR spectroscopy [131]. Brown seaweed, like *Ascophyllum nodosum*, *Laminaria, Fucus*, *Turbinaria* spp and *Sargassum* are the main source of current SE [76]. In this study, we investigate the effect of *Ascophyllum nodosum* on strawberry plants.

Nowadays, researchers are using SE as biostimulants for different purposes. In their review, Khan et al. [144] reported that SE promote in vitro propagation and protect the plant against pests and pathogens. Seaweeds contain growth regulators that improve plant growth, chlorophyll levels, root development, productivity, etc. [144, 146, 147]. For example, several studies showed the benefits of SE on growth of tomato seedlings grown under greenhouse condition [148] and root development of maize [149], grapes [150, 151], and winter rapeseed [152], which were grown under conventional regime. Besides, related to our study, treatment with seaweed extract significantly increased marketable fruit yield (by 8%) and root length (by 38%) of strawberry plants cv. Albion and Fortuna [147].

To enhance yield, Kocira et al. [153] reported the beneficial effects of the foliar application of SE on common beans. The use of SE can also promote the nutrient uptake of lettuce, tomato, winter rapeseed, and grapes [150, 152, 154, 155], resulting in the macro- (N, P, K, Ca, S) and micronutrients (Mg, Zn, Mn, Fe) accumulations [155]. From a commercial point of view, Roussos et al. [156] showed that SE improved marketability and had positive

effects on fruit size of conventionally grown strawberry without adverse impact on the pH of fruit juice, total soluble solid concentration, and acidity.

For strawberries, El-Minawy et al. [20] used a foliar spray of seaweed extract to increase plant growth, fruit yield, and quality of the strawberry cv. Sweet Charlie. Alam et al. [157] also showed that SE increased rhizosphere activities and microbial diversity of three cultivars of strawberry plants grown in a greenhouse and field. However, SE should be applied in proper conditions and appropriate concentration (4 g L^{-1} in the greenhouse and 1-2 g L^{-1} in the field) for having beneficial effects.

Today, two forms of SE are available for horticultural crops. The first form is farmyard manure which includes whole or chopped powdered algae, and the second form is liquid fertilizer (e.g. liquid seaweed fertilizer, seaweed liquid fertilizer, and liquid fertilizer) [158, 159].

1.5.2.4 Microorganisms

Microorganisms, as another type of biostimulants, play essential roles in the health of the plant and the soil. Microorganisms mostly found in the rhizosphere, the narrow volume of the soil which influenced by the plant roots [160]. They can be driven from bacteria (e.g. *Bacillus* spp., *Azotobacter* spp.) [121], yeast, fungi [107] such as *Trichoderma* spp. [161] and arbuscular mycorrhizal fungi (AMF) [162].

Microorganisms have several advantages on plant growth [163, 164] as well as on plant resistant to salinity [165], heavy metals, and other toxins [164]. They also improve macro- and micronutrient uptake via nutrients solubilization and nitrogen fixation, and productivity of crops by metabolic activity [121]. Furthermore, microorganisms are a beneficial component of the soil. They can have either a direct or indirect effect on the health of the soil [166]. Wani et al. [167] showed that microorganisms mediated mobilization of nutrients and mineralization processes of the soil, and nitrogen fixation in three cultivars of wheat [168].

1.5.2.4.1 Beneficial bacteria

As beneficial bacteria, plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting bacteria (PGPB) are prevalent free-living bacteria which are mainly isolated from the rhizosphere or plant roots [11, 169]. PGPRs are about 2-5% of the bacteria in the rhizosphere [160]. Other beneficial bacteria such as *Rhizobium, Bradyrhizobium,*

Azotobacter, Azospirillum, Pseudomonas, and *Bacillus* which are isolated from alkaline, saline, acidic, and arid soils can be used as biostimulants [17].

There are several positive impacts of beneficial bacteria on plant and soil health. As a type of biostimulants, beneficial bacteria can increase root growth, enhance mineral availability and nutrient use in the rhizosphere of crops [130, 170]. Studies like Backer et al. [171] showed that inoculation with plant growth-promoting rhizobacteria (PGPR) or treating with microbe-to-plant signal compounds could have a positive effect on crop growth. Additionally, beneficial bacteria help the uptake of specific nutrients through N fixation, P, or Fe solubilization [131]. As an example, Islam et al. [172] used proper beneficial bacteria for improving seedling vigor, root and shoot length, and dry biomass of tomato and red pepper under greenhouse conditions.

Some of PGPB increase the symbiotic relationship between plant roots and mycorrhizal fungi, which they called mycorrhiza helper bacteria (MHB) [131, 173]. In this case, according to Frey-Klett et al. [173] and Johansson et al. [174], MHP promote mycorrhizal fungus growth in the following mechanisms:

I) stimulation of fungal spore germination;
II) enhancing mycelial growth;
III) removal of toxins from the soil that prevent mycorrhizal growth or change rhizospheric chemistry or environmental effects to promote mycorrhizal growth;
IV) improving mycorrhizal root receptivity;
V) promote root branching by hormonal action;
VI) increased availability of nutrients such as N and P, thus improve synergy between mycorrhizal fungi and plant, which is required for both.

Recent plant agriculture relays on nitrogen fertilizer to maintain optimum yield, because nitrogen is a fundamental component of plant nucleotides, chlorophyll, and proteins [175]. However, these fertilizers are costly in terms of economy, and they have negative environmental effects and society health [176]. Fixing bacteria like *Azospirillum* are talking about the problems of fertilizers [176].

Nitrogen-fixing bacteria significantly increase chlorophyll content and uptake of macro- and micronutrients in tomato and red pepper [172]. *Rhizobium* and *Azospirillum* are two PGPB for a wide variety of plant species [177-180]. For instance, genetically modified

bacteria such as ammonium excreting *Azospirillum* improved the nitrogen (N) amount of wheat plants [181]. Among the twenty defined species of *Azospirillum* [182], *A. brasilense* and *A. lipoferum* are shown to have the highest performance [183, 184]. Therefore, we have chosen *A. brasilense* for our investigations. According to Malik et al. [185], *A. brasilense* and *A. lipoferum* contributed to the total nitrogen content of wheat (7-12%).

As other advantages of Azospirillum group as beneficial bacteria, Boddey et al. [186] showed that *Azospirillum diazotrophicus* could produce 60-80% of total nitrogen in sugarcane (*Saccharum officinarum*). Additionally, *Azospirillum* spp. can increase the nitrogen content in many plants, such as cotton and corn [11]. Furthermore, several studies reported the effects of *Azospirillum* on growth promotion [178], reduction of stresses, including salinity, drought [187-189], and heavy metal [190].

Beside *Azospirillum,* we also used bacterium called *Gluconacetobacter diazotrophicus*, which can fix atmospheric nitrogen [176]. In 2005, Suman et al. [191] reported that sugarcane is one of the main sources of this bacterium. They showed that *G. diazotrophicus* at lower levels of N cause plant growth and plant vigor of sugarcane. In addition, *G. diazotrophicus* survived in several crops such as corn [192] and sorghum [193] after inoculation. Furthermore, *G. diazotrophicus* used as biostimulant (rhizobial inoculated plants compared to uninoculated plants) significantly increased plant production and growth parameters of soybean [194] and maize [195]. As a result, nitrogen fertilizers can be reduced by applying plant growth-promoting bacteria [191].

Apart from nitrogen, phosphorus is another critical component for plants which has a limited plant availability in the soil due to processes such as precipitation, absorption, or conversion to the organic form. About 80% of phosphorus fertilizers cannot be up taken by plants [196]. Therefore, microorganisms with phosphate solubilizing capacity can help in supplying phosphates in an environmental-friendly and sustainable manner [197]. Here, *Pseudomonas* and *Bacillus* are two essential types of solubilizers for mineral phosphates.

In most cases, *Bacillus* and *Pseudomonas* commonly used as biocontrol agents. *Bacillus* spp. have the capacity to form spores which can survive in severe conditions [198]. As a result, these bacteria can potentially enhance the fertility of the soil and plant health [199]. More specifically, *Bacillus* spp. influence positively plant growth and excrete cytokinin in the rhizosphere [200]. Additionally, Arkhipova et al. [201] reported

that Bacillus spp. could improve cytokinin content of roots and shoots. Idris et al. [202] reported the positive effect of *Bacillus* on plant hormones (such as auxin), which influences plant development.

Other studies showed that Kiwi fruit growth could be improved by P- and K-solubilizing bacteria such as *Bacillus amyloliquefaciens,* XD-N-3, *Bacillus pumilus*, XD-P-1, *Bacillus circulans,* and XD-K-2 [203]. According to Mpanga et al. [204], the form of N supply is critical to increase plant growth by P-solubilizing microorganisms in maize. They report the use of stabilized ammonium instead of nitrate fertilization results in five strains of microorganisms on shoot biomass production, mineral nutrients like N, K, and Mn, enhance P acquisition, and stimulate the growth of the roots.

In general, beneficial bacteria applied directly to the soil or onto the seed by using peat, manure, compost or vermiculite as a carrier material [131]. It is worth noting that soil ecology is essential for proper inoculation, proper storage, and a good understanding of the local [205].

1.5.2.4.2 Beneficial fungi

Fungi are studied in a lot of research over the last decade because of their multi-level properties and success as biofertilizer [206]. With symbiosis, 90% of all plant species, mycorrhizal fungi are a heterogeneous group of microorganisms [10]. Arbuscular mycorrhizal fungi (AMF) is one of the abundant beneficial fungi in the plants which establish obligate symbiosis with over 75% of all vascular plants [207]. Due to many host plants, Arbuscular mycorrhizal fungi have several effects on plants and the environment.

AMF plays an essential role in plant P nutrition, growth, and performance, because of their capacity to enhance plant mineral uptake [114, 208]. Additionally, AMF is found to increase tolerance of the host plants to salinity by enhancing nutrient uptake, and generally abiotic stresses [209], improving photosynthesis [210] and preserving ion homoeostasis [211]. Researchers also showed that AMF can improve the quality of strawberries [212], tomato [213], and yam [214], by controlling secondary metabolites [215]. Boyer et al. [216] reported improvement in strawberry growth by increasing the root colonization of AMF and water use efficiency under deficit irrigation regime.

As a microbial biostimulant, *Rhizoglomus irregulare* and *Trichoderma* are two important beneficial fungi [114, 217]. *Rhizoglomus irregulare* and *Trichoderma* impact plant

development and growth [218]. Specifically, *R. irregularis* could increase nutraceutical quality of *L. barbarum* leaves in terms of rutin and acidic polysaccharide content [215]. Additionally, Lucini et al. [218] showed the inoculation of wheat roots by *R. irregularis* significantly increased the shoot dry biomass (18%), root dry biomass (39%), and root/shoot ratio (20%) compared to the control plants, while *Trichoderma* did not perform as well as *R. irregularis.*

Nevertheless, *Trichoderma* spp. are still one of the main fungi in sustainable agriculture because it was shown that they have biopesticide activity, they promote plant growth, and improve the nutritional quality and yield. They are also appropriate for abiotic stresses such as nutrient, saline, UV irradiation and oxidative stresses [161, 219]. Consequently, *Trichoderma* has important benefits in plant development and growth for horticultural, greenhouse, and field crops [217]. For example, Studholme et al. [220] reported that adding *Trichoderma* bran inoculum to the soil before sowing promoted root and shoot growth of cucumber and Arabidopsis. Shoot and root dry weights, and chlorophyll content were improved in the in vitro culture of tomato, melon, and pepper, and for greenhouse and field-grown lettuce and zucchini [221]. By combining three *Trichoderma* (*T. harzianum, T. viride, and T. virens*) Rudresh et al. [222] observed an increase of N and P uptake of chickpeas grown under glasshouse and field trials.

1.5.2.5 Organic acids (OA): as a new type of biostimulant

In this study, we proposed organic acids (OA) as another category of biostimulants which has low acidic characteristics. They are the third-largest category of biological products [223], and they cannot wholly dissociate with water [224]. Today, OA is used in different sectors like pharmaceutical products, food processing, petroleum, oil and gas production units [224].

Carbon skeletons and energy of the plant cells depend highly on organic acids. Also, the respiratory cycle and other biochemical processes use organic acids; as a result, they have a significant impact on the life of the plants and flowers [225]. Citric acid, lactic acid, and acetic acid are the most popular types of organic acids [224].

As a carboxylic acid, citric acid is widely distributed as an intermediate TCA (tricarboxylic acid) cycle in plants, animals, and microorganisms [226], which is a vital element of living systems and consumed products around the world [227]. In the tricarboxylic acid cycle, citric acid is directly involved in energy production as well as in some of the metabolism

mechanisms for specific amino acid, carbohydrates, and fatty acids [228]. Citric acid (and malic acid) can be applied in sustainable and organic plant production due to their effects on plant resistance against unfavorable conditions and yield enhancements [229].

Additionally, citric acids have positive impacts on the rooting response of 'Sherbet' roses when applied as foliar spray. Therefore, they increased the number of roots, the length of the root and shoot, and the biomass of the root and shoot dry [230]. In some of the studies, researchers used citrate and malate in plant roots to ease absorption of P and Fe from the soil [231]. Additionally, foliar spraying of citric acid with Fe sources are used to recover many plants from iron chlorosis [232, 233]. Other studies provided the following effects of citric acid on physiological responses:

I) increased essential oil production of sweet basil and dill [229];
II) enhanced the vase life of cut rose flowers [234];
III) improved nutrient absorption by roots of sweet basil [235];
IV) increased biomass and enhanced maximum fluorescence of sweet basil [236].

Beside citric acid, lactic acid is also a beneficial biostimulant, which can be produced by the fermentation as well as by chemical synthesis. About 90% of world lactic acid production is produced in the bacterial fermentation form [224]. According to Bohme et al. [237], foliar application of lactic acid can increase nutrient uptake, morphological parameters, and yield of vegetables such as tomato, cucumber, and French bean plants. They also showed that lactic acid increased leaf area of cucumber and bean plants, enhanced plastid pigment content, and net assimilation rate. Additionally, lactic acid improved tolerance of the plants to adverse growing conditions (temperature, pH, EC, and disease), and increased quantity and quality of crops yield [237].

1.5.3 Applying biostimulants

Biostimulants are mostly used to enhance plant and soil health. However, the application form is essential to have an optimal effect. Most of the biostimulants are applied in soil or growing media as powder, granules, or drench solution or via the irrigation system [153, 238]. Biostimulants can also be applied as seed treatment [130] or by foliar spray applications [239].

More specifically, biostimulants such as humic acids and nitrogen-containing products are mostly applied directly in the soil or as a foliar spray [153, 240]. Plant extracts, such as seaweed extracts are applied as seed priming, soil drenches, and foliar spray [76]. However, microbial inoculants are used in different ways. For instance, bacteria and fungi, in combination or alone, are applied directly to the soil (solid or solution) before plantation [241], as root drench [212, 242-244], foliar spray [245, 246], or seed dipping [247, 248].

Several studies showed the beneficial effects of foliar application of diverse biostimulants. Specifically, the foliar application of the humic acids allowed the plant to absorb nutrients quickly and directly by the grape leaf [249]. Humic substances contain carboxylate molecules and phenolate groups [250] which form complexes with several ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, and Fe^{3+}. Also, Tejada et al. [251] reported that foliar application of sewage sludge biostimulants (3.6 and 7.21 ha^{-1}) increased of the nutrients (macro and micro) levels in the maize leaves. Ferrara and Brunetti [252] reported that foliar application of humic compounds at different growth stages increased berry weight and grape quality parameters. However, the foliar application is dependent on various components of the cuticle, such as cuticular waxes, cutin, and cutan polymers.

The biostimulant mode of action may differ from one species to another due to the application form. Generally, the action of biostimulants starts when they enter the plant tissue [253, 254]. Biostimulants applied to the soil often impact the structure of the roots, resulting in an increase of the nutrient absorption by the plant. On the other hand, foliar extracts generally protect the plant from biotic and abiotic stress [255].

Several factors can affect the action of biostimulants. The leaf permeability differentiation between species [256], plant response characterizations, time of the application, and the optimal dose of usage could change the effectiveness of the biostimulants [238, 257, 258].

1.5.4 Biostimulants and organic farming

Plants which grow in organic agriculture are often suffering from nutrient deficiencies due to low soil nutrient levels or poor soil solubility of the nutrients. So, biostimulants may be an alternative method to solve some problems of organically-grown plants. In fact, they can contribute to increasing the availability of the nutrients as well as the cation exchange capacity of the soil (reduce the leaching of nutrients, particularly in sandy soils), supply nitrogen to the plants, and/or improve soil nutrient solubility [14]. In addition to nutrient deficiency, abiotic stresses may reduce yield by 50-70%. Therefore, biostimulants can

increase the resilience of the plant against these stresses and then may contribute to reducing the gap between organic and conventional crop yields [14, 259].

According to Tarantino et al. [260], the use of biostimulants in organic farming increased the quality attributes (dry matter, fruit weights, and soluble solid) of the pepper compared to the conventional system. However, the yield under conventional regime was higher than under organic fertilization management (without and with biostimulants). In another study, Amanda et al. [261] used biostimulant called Actiwave® (algal extract) with the concentration of 3 ml m^{-2} to reduce the nitrate content and to improve the commercial quality of baby leaf lettuce. They showed that biostimulants stimulated root growth, promoted the growth of plant and chlorophyll content of the lettuce leaves grown in the organic growing system.

Although biostimulants are very useful in organic farming, some of them are not registered for organic agriculture. For instance, organic farming prohibits the use of elicitor compounds which are synthetic chemical products [262]. As reported in several studies, among all biostimulants, natural biostimulants such as arbuscular mycorrhizal fungi (AMF) [263-265] and plant growth-promoting bacteria (PGPB) [266] are the famous one in organic horticulture. Olivares et al. [266] reported that tomato production in organic farming increased by foliar or soil application of PGPB and humate. Besides, Dorais [267] reported the beneficial effects of the use of microorganisms on nutrient availability such as N, P, and K and plant growth under organic conditions. Furthermore, researchers demonstrated the beneficial effects of biostimulants (e.g. plant-derived protein hydrolysate, AMF, humic acids, and N-fixing bacteria) on growth and development of the roots especially in organic farming [14, 268, 269]. In general, some of the biostimulants (e.g. *Glomus intraradices*, mycorrhiza) increase the soil aggregate and improve the availability of the nutrients under organic farming [270-272].

In addition to microorganisms, seaweed extracts also used in organic farming. According to Russo and Beryln [273], in the rain-fed plants, seaweed extract decreased the excessive use of fertilizers. It has also improved the absorption of the minerals by plants under organic agriculture. Additionally, in other studies, researchers reported the effects of seaweed extract applications on stimulation of rhizogenesis and root growth [274, 275].

These results motivated us to investigate the effects of different biostimulants on organic and conventional growing strawberries as few studies have compared the beneficial effect of biostimulants for organic strawberries compared to conventional ones.

2 MATERIAL AND METHODS

2.1 GREENHOUSE EXPERIMENT

This trial was performed in the high-performance greenhouse complex located at Laval University, Quebec, Canada (Lat. 46°78ʾ N; long. 71°28ʾ W).

Figure 2.2.1 The high-performance greenhouse complex located at Laval University, Quebec

The trial was carried out over six months from February 5th to July 11th, 2018. *Fragaria* × *ananassa* Duch. Cv. Monterey, a day-neutral cultivar, was used in this study. FIO Inc. (Île d'Orléans, Québec) provided tray plants. Each plant was placed in 1,9 L pots filled with standard substrate (BM4 40 NFW with 40% wood fibre and 60% peat) and organic substrate (OM4 40 NFW with 40% wood fibre, 50% peat, and 10% compost) provided by Berger (Saint- Modeste, QC, Canada) (www.berger.ca). Plants were grown under natural light supplemented with HPS lamps providing a PPFD of 162 µmol/m^2/s at the plant level, for a photoperiod of 16 hours (from 8 a.m. to 24 p.m.), with CO_2 concentration of 600-700 µL L^{-1}, day/night temperature 18/13 ± 0.8 °C and a vapor pressure deficit of 1.27 kPa. Bumblebees as a natural pollinator (Biobest®, Ontario, Canada) were used to improve flower pollination inside the greenhouse.

2.1.1 Treatments

A total of 14 treatments were compared under conventional (7 treatments) and organic (7 treatments) growing management. For the conventional growing system, the treatments consisted of 1- Conventional control, without biostimulant (CONTROL), 2- Seaweed extract (Acadian Seaplants Lte, Dartmouth, NS, Canada; SEAWEED), 3- *Trichoderma harzianum* strain T22 (TRICHO), 4- *Rhizoglomus irregulare* (Berger; MYC), 5- *Azospirillum brasilense* (free nitrogen fixator and denitrification), *Gluconacetobacter diazotrophicus* (endosymbiotic nitrogen scavenger), and *Bacillus amyloliquefaciens* (phosphate and potassium solubilizing bacteria) (Berger; BACT), 6- Mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria (treatments 4 and 5) (MYC+BACT), and 7- Citric acid-based formulation (Fungout®, pH=6.2, AEF GLOBAL Inc. , Lévis, Québec,Canada; CITRIC). For the organic growing system, the biostimulant treatments were: 8- Organic control without any biostimulants (CONTROL), 9- Seaweed extract (Acadian Seaplants Lte, Dartmouth, NS, Canada; SEAWEED), 10- *Rhizoglomus irregulare* (MYC), 11- *Azospirillum brasilense* (free nitrogen fixator and denitrification), *Gluconacetobacter diazotrophicus* (endosymbiotic nitrogen scavenger), and *Bacillus amyloliquefaciens* (solubilizer/ phosphate and potassium mineralizator) (BACT), 12- Mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria (treatments 10 and 11) (MYC+BACT), 13- Combination of mycorrhiza and nitrogen-fixing endosymbiosis bacteria with only a basic fertilization (MYC+BACT/LF), and 14- Citric acid-based formulation (Fungout®, pH=6.2, AEF GLOBAL Inc., Lévis, Québec, Canada; CITRIC).

Seven hundred liters of the modified standard substrate (BM4 40 NF Wood with 40% wood fibre + 60% peat) and organic substrate (OM4 40 NF Wood with 40 % wood fibre + 50% peat + 10 % compost) were used for both growing systems. Two commercially available products were selected as biostimulants: *Ascophyllum nodosum* (rockweed) seaweed pure extract (Acadian Seaplants Lte, Dartmouth, NS, Canada), and citric acid-based formulation commercially available as Fungout® (AEF Global, Lévis, Québec, Canada). Seaweed extract was applied to the substrate twice a month during the trial at a concentration of 0.4%. Citric acid (Fungout®) was sprayed to the aerial part of the plants twice a month at a concentration of 1.25% using a hand sprayer on the leaves and green fruits until runoff. Berger based-biostimulants (nondisclosure formulation) were added to the growing media before the plantation.

Plants were irrigated with liquid organic (0.3% of Nature's Source (3-1-1) and 0.00035% of potassium silicate) or synthetic fertilizers (Table 2.1). Silicate, the most abundant elements in the soil, may enhance the growth, development, yield and the quality of the organically grown strawberries. Potassium silicate was used as a source of silicon and potassium in our experiment [276, 277]. However, this product is not allowed for organic farming in Canada. A drip irrigation system by using pressure compensating drippers connected to sprinkler stakes was used for the conventional and organic growing systems. Plants were fertigated three times per day, with a duration of three minutes. The amount of nutrient solution was 360 mL day^{-1}. To adjust the nutrient solution, the volume, pH, and electrical conductivity (EC) of the nutrient solution and drainage solutions were measured daily. For the organic growing system, 5.5 g of poultry manure pellets (5-3-2; Acti-sol Inc. Quebec, Canada) were applied to the organic growing plants twice a month except in treatment number 13. Plants were daily irrigated with water and some nutrients via a drip irrigation system providing 360 mL day^{-1}.

Table 2.1 Mineral elements and their concentration in the conventional and organic management in the greenhouse.

Conventional management				**Organic management**	
Macro nutrients	**Concentration ppm**	**Micronutrients**	**Concentration ppm**	**Nutrients**	**Concentration ppm**
Potassium silicate	70000	Fe	11	Nature's Source (3-1-1)	3000
Calcium nitrate	290	Mn (Manganese 13%)	3	Potassium silicate	3.5
Mono Potassium Phosphate	240	Zn (35% zinc sulphate)	1		
Mg Sulfate	200	B (Borax 15%)	1		
Potassium nitrate	183	Cu	1		
Potassium sulphate	30	Mo (Sodium molybdate)	0.015		
Ammonium nitrate	22.5				

2.1.2 Experimental design

The experimental design was defined as a randomized complete block design with five replicates. Three hundred fifty pots of 1.9 L (155 mm ×150 mm) were then arranged randomly into five complete blocks of 70 pots per block and five pots per experimental unit (Figure 2.2). As shown in Figure 2.2, 35 experimental units were organic treatments, displayed in blue color, and 35 experimental units were conventional treatments, represented in gray color. The guard plants showed by the red color in the side of each repetition.

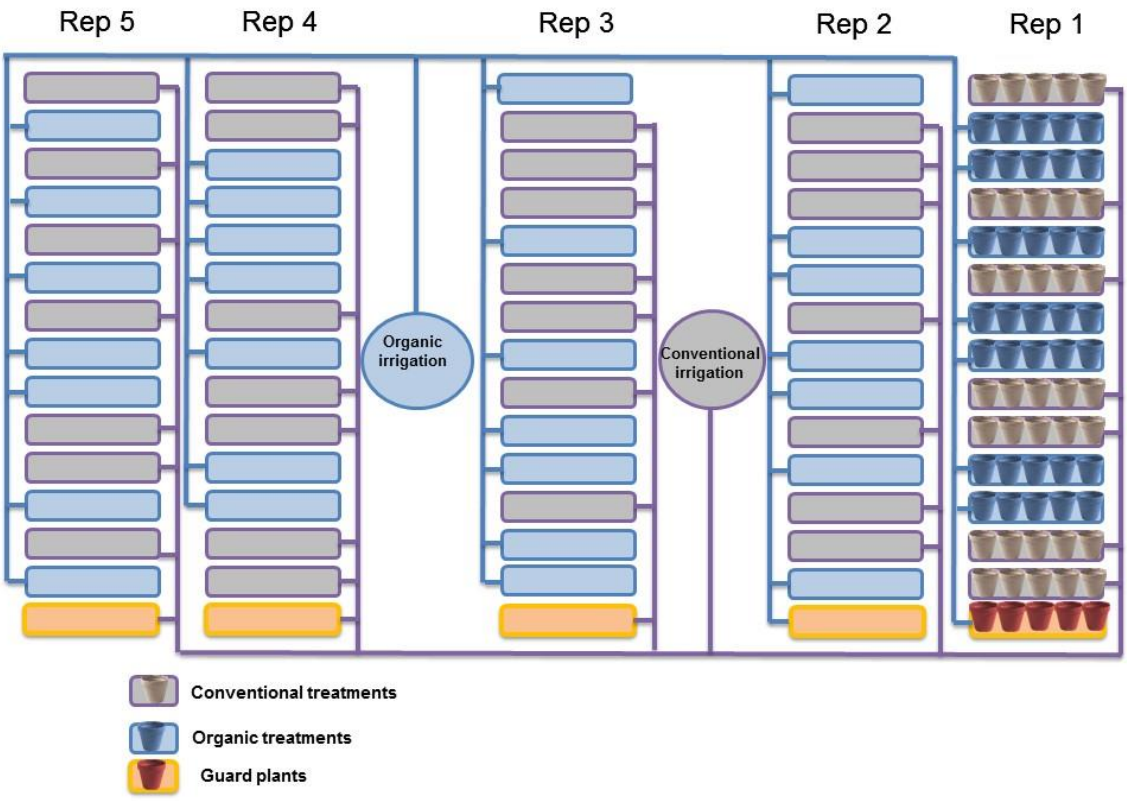

Figure 2.2 Experimental design in the greenhouse experiment (winter 2018).

2.2 HIGH TUNNEL EXPERIMENT

2.2.1 Growing conditions

The high tunnel trial was conducted at the farm Les Fraises de l'Ile d'Orleans Inc. (St. Laurent, Ile d'Orleans, Quebec, Canada, Lat. 46° 51.789285' N; long. 71° 1.57608' W) in 2018 from May 10th to October 2nd. This experiment aimed to validate the effects of five biostimulants on yield and quality of conventional strawberry crops under soilless and high tunnel growing system. Strawberry plants were cultivated in high tunnels of 4.8 m high, 91.4 m length, and 8.4 m width per bay covered with a simple polyethylene plastic membrane. The sides of the tunnel were opened to allow ventilation. Before transplanting, mounds (40 cm tall, 25 cm width and 15 cm depth) were prepared and covered with the tight black plastic film. Drainage was deposited at the bottom and covered with plastic mulch. The beds were then filled with peat-based growing media (BM4 40 NF Wood 25 with 25% wood fibres and 75% peat; Berger Peat Moss, Saint-Modeste, Canada) providing 2,28 L per plants. This soilless growing system avoids any direct contact with the native soil, which is contaminated with *Verticillium* spp. and other soil-borne pathogens. Strawberries were planted at the distance of 20 cm on a double row with zigzag form (staggered) with the density of 10 plants per linear meter (56250 plants per ha) and 60 plants per experimental unit. We used day-neutral 'Monterey' cultivar strawberry tray plants (*fragaria* × *ananassa* Duch.) provided by FIO Inc. Disease and insect control practices were carried out during the growing period (annex A.1). Bumblebees as a natural pollinator (Biobest®, Ontario, Canada) were used to improve pollinate plants.

Figure 2.3 The high tunnel of Les Fraises de l'Ile d'Orleans Inc. (St. Laurent, Ile d'Orleans, Quebec, Canada).

Air and substrate temperature and relative humidity of the substrate were recorded every 15 minutes, with HOBO sensor recorders (Onset computer corporation, Bourne, MA, USA). The HOBO was protected by a solar radiation shield and installed in the experimental unit. A drip irrigation system was ensured the fertigation of the plants. The irrigation pipes were placed in the middle of each row at a rate of 9.8 holes/ linear meter. Plants were irrigated once or twice a day with synthetic fertilizers for a volume of 700 mL per irrigation per plant (Table 2.3).

Table 2.1 Mineral elements and their concentration used for vegetative and flowering and fruiting plants grown in the high tunnels.

	Vegetative		Flowering-fruiting			Vegetative		Flowering-fruiting	
Macro-nutrients	**mol/L**	**ppm**	**mol/L**	**ppm**	**Micro-nutrients**	**µmol/L**	**ppm**	**µmol/L**	**ppm**
NH_4	0.4	6.0	0.0	0.0	Fe	25.0	1.4	25.0	1.4
K^+	4.3	166.5	3.4	134.1	Mn	18.0	0.99	18.0	0.99
Ca^{++}	1.6	65.4	1.0	38.0	Zn	8.5	0.4	8.5	0.4
Mg^{++}	1.2	27.7	0.6	15.0	B	12.0	0.2	12.0	0.2
NO_3.	5.4	76.2	3.9	54.2	Cu	8.0	0.3	8.0	0.3
$H_2PO_4^-$	2.4	74.5	1.1	34.0	Mo	0.5	0.01	0.5	0.01
SO_4^{--}	1.1	36.7	0.6	19.9	Fe	25.0	1.4	25.0	1.4

2.2.2 Treatments

A set of six treatments 1- Control without biostimulant (CONTROL), 2- *Rhizoglomus irregulare* (MYC), 3- *Azospirillum brasilense* (free nitrogen fixator and denitrification), *Gluconacetobacter diazotrophicus* (endosymbiotic nitrogen scavenger), and *Bacillus amyloliquefaciens* (solubilizer/ phosphate and potassium mineralisator) (BACT), 4- Mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria (MYC+BACT), 5- Citric acid-based formulation (Fungout®, pH=6.2, AEF GLOBAL Inc. , Lévis, Québec, Canada; CITRIC), and 6- Citric and lactic acid-based formulation (Tivano™, pH=4.8, AEF GLOBAL Inc., Lévis, Québec, Canada; CITRIC+LACTIC) were used.

Fungout® and Tivano™, two commercial products, were sprayed to the aerial part of plants twice a month with a concentration of 1.25%. Mycorrhiza and bacteria were added once to the growing media before the plantation (non-disclosure formulation).

2.2.3 Experimental design

The experimental design was a complete randomized block design with four replicates. Twenty-four experimental units were randomly arranged into four complete blocks. The length of each experimental unit was six meters and a space between each block was two meters (Figure 2.4). Each replicate was in the different bays.

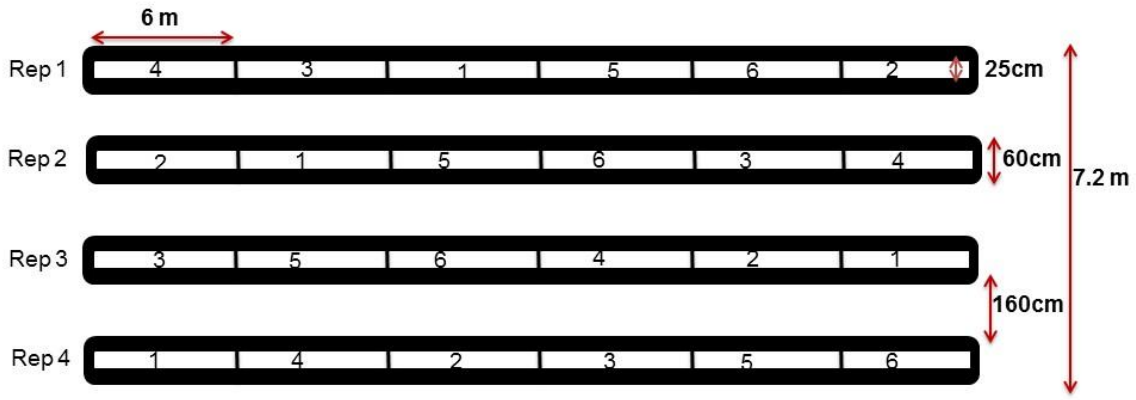

Figure 2.4 Experimental design in the high tunnel experiment during summer 2018

2.3 MEASURED PARAMETERS

2.3.1 Soil activity

2.3.1.1 Fluorescence diacetate hydrolysis (FDA)

Fresh soil composite samples were prepared to determine the soil biological activity based on the total microbial population. For each experimental unit, in the greenhouse and high tunnel experiments, total of 30 g of soil was sampled (3 subsamples from each experimental unit) just below the surface using a trowel. In the greenhouse experiment, soils were sampled on 09th May and 11th and 19th June. In addition, soils were sampled on 13th and 24th September, 13th July and 24th October in high-tunnel. The trowel was disinfected with 70% alcohol between each sampling to avoid contaminating the samples. Sampling was performed in the morning before first irrigation. There were thus 4 and 5 replicates for each treatment in the high tunnel and greenhouse, respectively. Soil samples were stored at 4°C for a maximum of one to two days before analysis.

Biological activity was determined by the hydrolysis of fluorescein diacetate (FDA), which measures enzymatic activity produced by several microorganism enzymes [278]. Briefly, 0.1 g of fluorescein diacetate (3'6'-diacetyl-fluorescein., Sigma®, F7378-100G) dissolved in 80 mL of acetone (Certified ACS, Fisher Chemical) under a chemical hood. Then, the volume was adjusted to 100 mL with acetone. The solution was stored in the -20°C. Also, 60 mM potassium phosphate buffer solution was prepared and stored in the 4°C. The pH of the solution was 7.6. The ingredients of the buffer consist of 8.7 g di-potassium hydrogen orthophosphate (K_2HPO_4, Anachemia VWR Company, Canada), 1.3 g

potassium dihydrogen phosphate (KH_2PO_4, Anachemia VWR Company, Canada), 800 mL of demineralized water, and 200 mL deionized water.

Consequently, the corresponding standard solution with a concentration of 2000 µg mL^{-1} was prepared and stored in the dark for a maximum period of one week. In order to prepare a standard solution, 0.2265 g of fluorescein sodium salt (Sigma®, F6377-100G) was added to 100 mL of 60 mM potassium phosphate buffer. After making the solutions, 2 g of fresh soil was weighed into 50 mL falcon tubes with three replicates. Then, 30 mL of 60 mM potassium phosphate buffer was added in the tubes. In parallel, the blanks were prepared. The enzymatic reaction was initiated by adding 600 µL mL^{-1} of a 1000 µg fluorescein diacetate solution to each sample tube. Then, tubes incubated and shacked with 200 rpm at 30°C for 20 minutes. After this step, tubes were centrifuged at 4500 rpm for five minutes. In parallel, a standard curve (0, 1, 3, 5 and 10 µL mL^{-1}) made by diluting a 0.5 mL of the solution of 2000 µg mL^{-1} fluorescein in a 49.5 ml 60 mM potassium phosphate buffer. The hydrolysis of the FDA was measured 40 minutes after the beginning of the reaction at 490 nm using a spectrophotometer (BioTek Instruments, Inc. Epoch 2 Microplate reader). A more pronounced yellow color indicated a higher enzymatic activity and consequently a higher microbial activity of the sample.

2.3.1.2 Metagenomic analysis of soil DNA

Metagenomic analyses evaluated bacterial, eukaryotic, and fungal diversity. Soil samples were collected in the morning before the first irrigation on 18th June, as described in the previous section. We have selected only few treatments in the greenhouse experiment which treated with microorganisms to identify the metagenomic analysis. For selected treatments, there were 3 replicates. Soil samples were stored in the -20°C until further analysis.

An amount of 0.5 g of substrates was prepared and used for DNA extraction. The commercial FastDNA Spin Kit for Soil Extraction Kit (MP Biomedicals, Solon, OH, USA) with a FastPrepR- 24 (MP Biomedicals, Solon, OH) homogenization step was used according to the manufacturer's instruction. The quality and quantity of the extracted genomic DNAs were determined spectrophotometrically with the absorbance measurements at 260 nm and 280 nm and the A260 / A280 ratio. The primer sequences of the specific regions were used to the amplification of the V6-V8 regions of bacterial 16S, 18S rRNA of eukaryotes and the fungal ITS1 region as described by Comeau et al. [279]

and McGuire et al. [280]. A dual-indexed PCR approach was used to complete library preparation. This approach specifically designed for Illumina instrument, developed by the genomic analysis platform IBIS (Laval University, Quebec City, Canada). The amplicon libraries were sequenced in paired-end format with a reading of 300 bases, 2x300 base pairs on each side of the DNA strand on Illumina MiSeq at the genomic analysis platform [281].

2.3.1.3 CO_2 efflux

Soil respiration was determined from CO_2 fluxes emitted at the soil surface of one plant per experimental unit (total of five plants per treatment). CO_2 flow measurements were taken at the end of the greenhouse experiment on 18th June (163 days after plantation) by using a portable gas exchange measurement system, model LI-6400 (Li-Cor Biosciences, Lincoln, Nebraska, USA), and a chamber for ground breathing, model 6400-09. The measurements were taken in the morning before the first irrigation to obtain representative flows of the soil respiration. Once the device turned on, the chamber was hermetically affixed to the collar. A soil thermometer was also inserted into the substrate and gave the soil temperature in real-time. The device measured two or three measurement cycles which then allowed to calculate an average flow per experimental unit. All subsequent steps were performed following the procedure issued for this type of device in the “Using the LI-6400” and “Using the 6400-09 Soil Chamber” reference manuals (LI-COR, Inc., 2012). The data was then exported from the device to a computer in Excel format.

2.3.2 Physiological parameters

2.3.2.1 Chlorophyll fluorescence

Chlorophyll fluorescence (ChlF) analysis was performed by using a Handy PEA fluorimeter (Handy Plant efficiency analyzer, Hansatech Instruments Ltd., King’s Lynn, UK). The chlorophyll fluorescence was measured monthly on 05th March, 02nd April and 04th May in the greenhouse experiment and on 04th July, 06th August and 06th September in the high tunnel experiment.

The measured leaves were dark-adapted for 20 minutes by attaching light exclusion clips to the leaf surface, avoiding the central vein, while the plants were in the light. The Fv (ratio of variable fluorescence), Fm (maximum fluorescence value), maximum Fv/ Fm (the maximum quantum efficiency of photosystem II), and Performance Index (indicator of

sample vitality) parameters were recorded for one second with 3000 μmolm^{-2} s^{-1} PPFD (Photosynthetic Photon Flux Density). For each experimental unit, at least three plants were randomized selected and measurements performed and recorded (3 plants with one leaf reading per plant; 15 plants for the greenhouse experiment and 12 plants for the high tunnel experiment).

The parameters were calculated by equations described by Strasser et al. [282].

Equation 2.1

$$F_v/F_m = (F_M - F_0)/F_M$$

Equation 2.2

$$PI = \frac{1-(F_0/F_M)}{M_0/V_J} \times \frac{F_M - F_0}{F_0} \times \frac{1-V_J}{V_J}$$

Where F_0= fluorescence intensity at 50 μs; F_J = fluorescence intensity at the J step (at 2 ms); F_M = maximal fluorescence intensity; V_J = relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_0)/(F_M - F_0)$; M_0 = initial slop of fluorescence kinetics, which can be derived from the equation:

Equation 2.3

$$M_0 = 4 \times (F_{300\mu s} - F_0)/(F_M - F_0).$$

2.3.2.2 Chlorophyll content

The leaf chlorophyll content was measured using chlorophyll meter (SPAD-502, Minolta Corp.) monthly on 02nd March, 02nd April and 02nd May in the greenhouse experiment and from 16th July, 16th August and 13th September in the high tunnel experiment. The chlorophyll content was determined by the average of three reading per leaf for a total of 9 measurements per experimental unit (3 plants with 3-leaf reading per plant). Chlorophyll content was estimated in the same leaf where chlorophyll fluorescence was measured.

2.3.2.3 Photosynthesis

Leaf photosynthesis light-response curves were carried out on one plant per experimental unit on 29th , 30th and 31st May and 1st June, for the greenhouse experiment and on 11th , 12th and 13th July for high tunnel experiment using a portable gas exchange system, model LI-6400 (Li-Cor Biosciences, Lincoln, Nebraska, USA). Gas exchange was measured on

the highest leaf of one plant per experimental unit. Measurements were performed in the morning one hour after irrigation. Briefly, measurement system was set at 1800 µmolm^{-2} s^{-1} Photosynthetic Active Radiation (PAR), an air temperature of 24°C, a Vapour Pressure Deficit (VPD) of 1.3 kPa, a leaf chamber CO_2 concentration of 450 µmol mol^{-1}, and flow rate of 350 µmol s^{-1}. After around 15 minutes acclimation period, the intensity of light was varied from high to low PAR (1800, 1500, 1200, 900, 700, 550, 375, 275, 200, 150, 100, 75, 50, 20, and one µmol photons m^{-2} s^{-1}), and gas-exchange parameters were recorded for each light level. Then, photosynthesis parameters such as dark respiration rate (Rd), light compensation point (LCP), quantum efficiency (Φ), and maximum rate of photosynthesis (Amax) were extracted from the curves as described by Hansen et al. [283].

2.3.3 Non-destructive growth parameters

Plant growth was measured monthly on the three random samples of strawberry plants per experimental unit on March 02nd, April 01st and May 02nd in the greenhouse and 16th July, 16th August and 13th September in the high-tunnel. The measured parameters included the number of leaves, number of flowering fruit stalks, number of crowns, as well as the diameter of crowns. Leaves, flowering fruit stalks, and crowns were counted and recorded. The diameter was measured using a digital caliper (Neoteck 6 inches, Hong Kong) and recorded. Specifically, measurements were performed in March, April, and May for the greenhouse experiment and in July, August, and September for the high tunnel experiment.

2.3.4 Destructive growth parameters

2.3.4.1 Fresh and dry biomass

Three plants per experimental unit were cut off from the collar at the end of the experiment (on 13th June for greenhouse experiment and on October 1st for high tunnel experiment). For both experiments, leaves, stems, and flowers of the plant were weighted. For the greenhouse experiment, roots were washed carefully with water to remove substrate particles, and the fresh matter was weighed and recorded. Then, put in the well- defined paper bags and dried at 60 °C for two weeks. After two weeks**,** dry matter of aerial and root parts was weighed and recorded.

2.3.4.2 Leaf area

The leaf area of the leaves for each plant was determined at the end of the greenhouse experiment period (on 13th June) using a planimeter (Li-3100c Area Meter Model, Li-COR, 66 Nebraska, USA). Each leave was inserted into the planimeter previously calibrated with a calibration disc to determine the total leaf area per plant.

2.3.5 Foliar mineral analysis

Leaf sampling was performed for both experiments from each experimental unit to determine their mineral content (N, P, K, Mg, Ca, Mn, Mo, Na, Al, Si, Zn, and S). Specifically, sampling was performed on 29th March and May 01st for the greenhouse experiment and on 09th July and 13th August for high tunnel experiment. Three fully developed leaves from three plants per experimental unit were sampled. The concentrations of nutrients were determined based on percent or ppm of the dry matter. Samples were put in well-identified paper bags and dried at 60°C for 48 hours. Dry matter was crushed to small parts using the grinder (Cuisinart, China) and stored in the vials to performing the foliar mineral analysis by method described by du Québec, C.D.P.V [284].

2.3.6 Yield

Fruit yield was evaluated once or twice per week for the greenhouse experiment and three times per week for high tunnel experiment during fruit harvest. Specifically, in the greenhouse experiment, the first harvest was on March 27th and the last harvest was on June 11th. Besides, for high tunnel experiment, June 23rd and October 2nd were the first and last dates of harvest, respectively. At each harvest, a fruit classification was performed according to the shape and size of the fruits. Fruits were then classified into two groups: marketable and unmarketable fruits. For each treatment, the number and weight of the fruits were recorded. The number and weight of the marketable and unmarketable fruits were the measured parameters. A fruit was considered unmarketable when smaller than five grams and 1.90 cm as well as signs of diseases and poor pollination.

2.3.7 Fruit quality

2.3.7.1 Total sugar level (°Brix)

Soluble sugar content (SSC) or °brix is a sweetness measurement, and it was measured using a refractometer (Atago PAL-1 (3810)). °Brix was evaluated twice a month on both experiments (on 01st, 15th April, 01st and 15th May) for the greenhouse experiment and on

01st, 15th and 29th July, 12th and 26th August, and 09th September for high tunnel experiment). Fully ripe fruits were harvested at the day of measurement. Three ripe fruits from each experimental unit were selected to make the °brix measurement. Fruits were crushed using a blender or garlic press, and then the pulps and seeds were removed by using a filter paper. Few drops of this sample juice were placed on the refractometer by using a plastic pipette to record the %SSC or Brix value. Between each reading, the refractometer was cleaned with distilled water and calibrated to 0% SSC at the beginning of each measurement.

2.3.7.2 Total phenolics assay (Folin-Ciocalteu method, TPC)

Ten fruit samples were collected at optimum maturity for polyphenols in April and May (on 01st, 15th April, 01st and 15th May) for the greenhouse experiment and on 01st, 15th and 29th July, 12th and 26th August, and 09th September for high tunnel experiment and stored at -20 °C until analysis. Total phenolic content was measured using the Folin-Ciocalteu (F-C) method described by Singleton and Rossi [285]. Briefly, seven freeze-dried fruits per experimental unit were freeze-dried (model of the lyophilisator) and homogenized by using a coffee grinder. Folin-Ciocalteu reagent, sodium carbonate solution, gallic acid, and methanol 80% were prepared. Then, solid samples were extracted from the powder. For each sample, 0.3 g of powder was added into a 50 mL falcon tube with three replicates. Then, we mixed the powder with 20 mL of methanol 80% and placed in the ultrasonic bath at 37 °C for 20 minutes. After centrifuging at 4000 rpm for 4 minutes, we transferred the supernatant to another 50 mL falcon tube. The extraction was repeated three times, with 20 mL of methanol 80%. Then, the extraction was completed with water. In parallel, the standard solutions were prepared. After diluting liquid extraction, 20 µL of water (white extracts), sample, and standard were mixed with 100 µL of Folin-Ciocalteu reagent to realize the reaction. The processing time was 1-8 minutes. Then, the amount 80 µL of the 7.5% sodium carbonate solution was added (Na_2CO_3) to a 2 mL vial. Next, it was mixed well and allowed to stand for 45 minutes. Absorption was measured at 765 nm using a Spectrophotometer (BioTek Instruments, Inc. Epoch 2 Microplate reader).

2.3.7.3 Anthocyanin

Ten ripe fruit samples per experimental unit were collected monthly (on 01st, 15th April, 01st and 15th May) for the greenhouse experiment and on 01st, 15th and 29th July, 12th and 26th August, and 09th September for high tunnel experiment) and stored at -20 °C until the anthocyanin analysis. The anthocyanin content of fruits was determined by the pH

differential method developed by AOAC International [286] and approved by Lee et al. [287]. Fruits were freeze-dried and their powders were extracted using methanol/water/ acetic acid (85: 15: 0.5 v/v, MeOH/ H_2O/ AcOH) previously reported by Wu and Prior [288]. In brief, 0.3 g of the sample powder was placed in 50 mL falcon tubes with three replicates. Then, two reagents were prepared: pH 1.0 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4 M). In the next step, 5 mL of the acidic methanol solvent was added to the tubes and well mixed by using a vortex for 30 seconds. Then, the tubes were placed in the ultrasonic bath for 15 minutes. Afterward, all the tubes were transferred to the centrifuge for 5 minutes at 4000 rpm. After centrifugation, the supernatant was transferred to the 50 mL falcon tube. In order to have homogenized extract, the extraction was repeated three times. Then, the test solution was prepared using pH 1.0 and pH 4.5 buffers to determine an appropriate dilution factor. After diluting the extracts, blanks were made with pH 1.0 and pH 4.5 buffers. At the last step of preparation, the amount of 0.5 mL of diluted extract and 2.5 mL of the buffers were added in the 4 mL cuvettes. The solution was mixed well and stands for 30 minutes in the room temperature. Samples were measured in the absorbance of 510 nm and 700 nm using spectrophotometer (BioTek Instruments, Inc. Epoch 2 Microplate reader). The difference in absorbance between the two samples was calculated using the following equation:

Equation 2.4

$$\begin{aligned}\text{Absorbance} = {} & [\text{A510nm(pH1.0)} - \text{A700nm(pH1.0)}] \\ & - [\text{A510nm(pH4.5)} - \text{A700nm(pH4.5)}]\end{aligned}$$

The concentration of the anthocyanin was calculated by cyanidin-3-glucoside equivalents as follows:

Equation 2.5

$$\%\,{}^{w}/_{w} = \frac{A}{\varepsilon \times L} \times MW \times DF \times \frac{V}{Wt} \times 100$$

Where, A= absorbance; ε= 26 900 molar extinction coefficient, in L ´ mol–1 ´ cm–1, for cyd-3.glu; L= pathlength in cm; MW (molecular weight)= 449.2 g/mol for cyanidin-3.glucoside (cyd-3.glu); DF= dilution factor established in D; V= final volume of the solvent; Wt= weight of the sample.

2.4 STATISTICAL ANALYSIS

All data were analyzed by a two-way model of analysis of variance (ANOVA) by using the MIXED procedure of SAS software (version 9.4, SAS Institute Inc. Cary, NC). Before the analysis data, the test of normality was performed, and the appropriate transformation (arcsine square-root or log) was used. In greenhouse and high tunnel experiments, for parameters with repeated data, repeated MIXED procedure in time was used to evaluate the effect of treatments and time on growth, yield, and quality. Treatments and time variables (date or weeks) were considered as fixed effects, block as a random effect, and the group of plants of each experimental unit was the repeated measure. For each variable, the MIXED procedure of SAS (SAS Institute Inc., 2013) was used with appropriate repeated statements and covariance structures that minimized the Akaike criterion. For variables without repeated measures, the same model and MIXED procedure were used to study the effect of treatments on growth, yield, and quality. Also, the normality of data was checked using the Shapiro-Wilk statistic, and homogeneity of variance was assessed visually by examining the graphic distribution of residuals.

Furthermore, protected Fisher's LSD (least significant difference) was used for pairwise comparisons. Treatment and time effects were considered significant at a 5% confidence level ($P \leq 0.05$). A contrast statement was added to compare overall the effects of treatments used in organic management and treatments used in conventional management on all variables. Principal component analysis (PCA) was conducted on leaf mineral concentration, physiological, yield, quality, and soil activity parameters. Spearman correlations were tested in order to find links between variables without the influence of extreme data measurements.

3 RESULTS

In the present study, we have investigated the effect of biostimulants on plant development, yield, and fruit quality of strawberry plants. In this chapter, first, the results on the effects of plant biostimulants on soil microbiota in the greenhouse experiment conducted in winter 2018 are shown (section 3.1.1) followed by plant photosynthetic performance (3.1.2), plant growth (3.1.3 and 3.1.4), foliar mineral concentrations (3.1.5), yield (3.1.6) and fruit quality (3.1.7). Then, high tunnel experiment results performed in summer 2018 are presented (section 3.2) regarding soil microbial activity (3.2.1), photosynthetic performance (3.2.2 and 3.2.3), plant growth (3.2.4), foliar mineral concentrations (3.2.5), yield (3.2.6), and fruit quality (3.2.7).

3.1 GREENHOUSE EXPERIMENTS- WINTER 2018

3.1.1 Soil activity

3.1.1.1 Fluorescence diacetate hydrolysis (FDA)

Regardless of the biostimulants, soils of the organic growing system showed a significant (P<0.0001; +66%) increase in microbial activity compared to the conventional one (Figure 3.1). For both growing systems, biostimulant treatments did not influence the microbial soil activity, expressed by the hydrolysis of the fluorescence diacetate, compared with its respective control. However, the low fertilization treatment (MYC+BACT/LF) under the organic crop management decreased the soil microbial activity (37%) compared with the organic control (Figure 3.2).

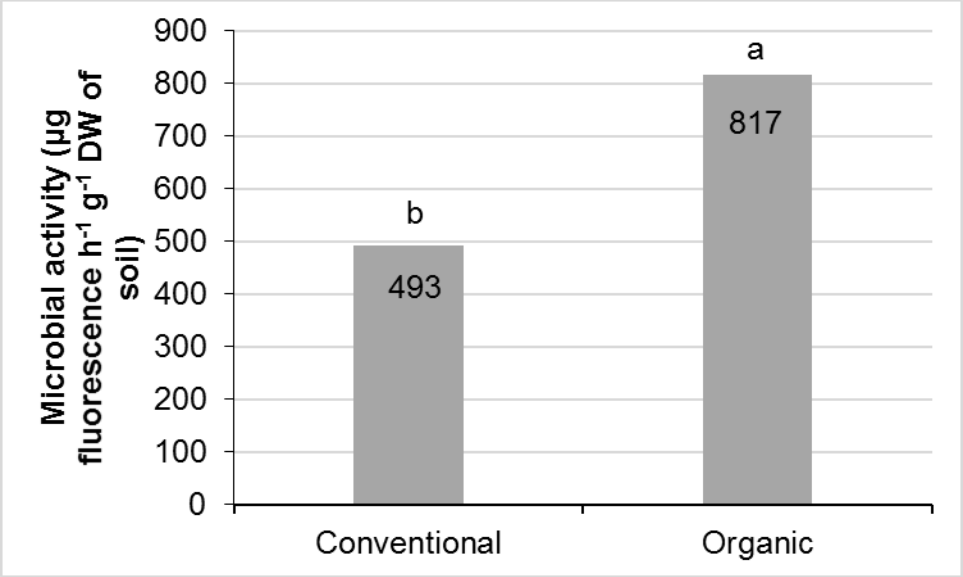

Figure 3.1 Variations of microbial activity of the strawberry plants by growing system during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05). Microbial activity expressed by µg fluorescence h^{-1} g^{-1} dry weight of soil (See annex B.1 for data and P values).

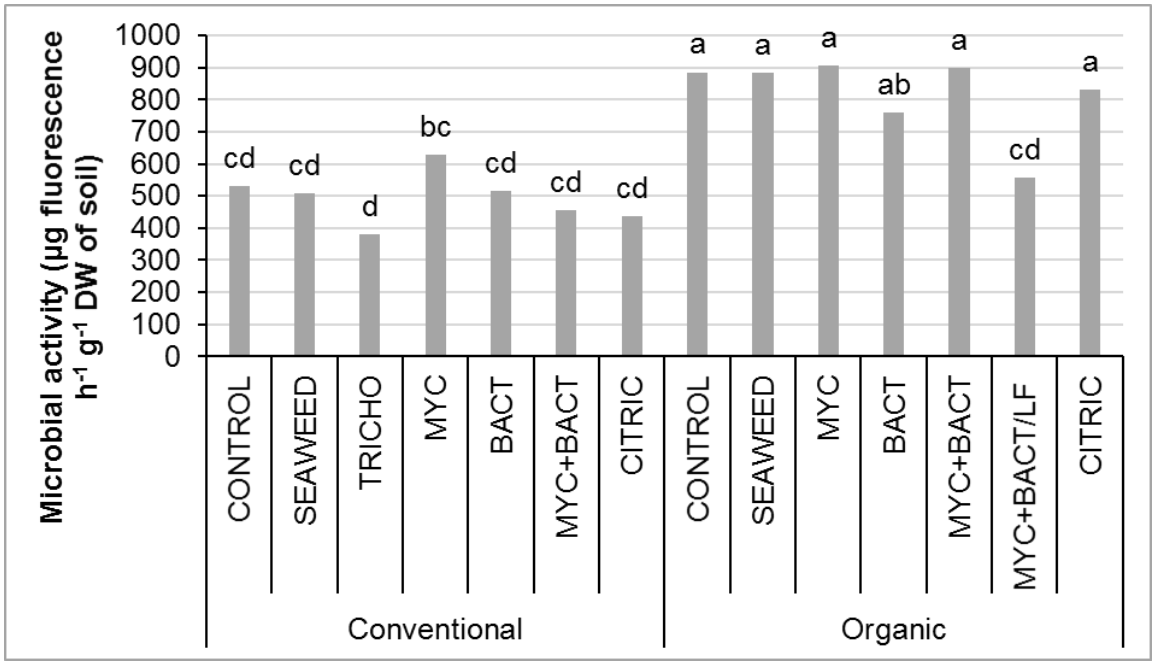

Figure 3.2 The influence of studied biostimulants on microbial activity of the soil during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05). Microbial activity expressed by µg fluorescence h^{-1} g^{-1} dry weight of soil (See annex B.1 for data and P values).

3.1.1.2 Metagenomic analysis of soil DNA

The determination of the microorganism abundance and composition were performed for the control, mycorrhiza, bacteria and the mixture of mycorrhiza and bacteria treatments for conventional and organic growing systems in two replicates.

Figure 3.3 clearly shows that soil bacteria and fungi abundance were higher under organic growing management compared with the conventional cultivation. The ratio of bacteria to fungi was also higher under organic cultivation. However, the studied biostimulants did not increase the soil abundance of bacteria and fungi compared with its respective control.

The diversity of the dominant bacteria, fungi, and eukaryote expressed as the Shannon index is shown in Figure 3.4. Our results showed that the diversity of all groups was higher in the conventional compared to the organic samples, except for the bacteria diversity of the conventional control and the MYC+BACT, which were similar (Figure 3.4a). Although large variations were observed, the studied biostimulants increased the bacterial diversity of the conventional soil samples compared with control (Figure 3.4a), while the combination of bacteria treatment increase the diversity of the fungi (Figure 3.4b). For the organic soil samples, the combination of mycorrhiza and bacteria increased the bacterial diversity compared with the control, while no effect was observed for the fungi (Figure 3.4a). The diversity of the eukaryotic groups in the organic soil samples was, however, increased by the biostimulant treatments (Figure 3.4c).

The results of the PCoAs (Figure 3.5) clearly showed that the microbial soil composition was strongly influenced by the growing system. Indeed, the data of bacteria, fungi, and eukaryotes for conventional soil samples are mostly located in the one left quadrate, while the organic soil samples are in the two right quadrates. In the PCoA of bacterial communities of the growing medium (Figure 3.5a), 8.4% of the variation for conventional and organic samples was explained by the vertical axis and 23.8% by the horizontal axis. For the fungi communities, the vertical axis explained 13.8% of the variability, while the horizontal axis represented 43.8% of the variability (Figure 3.5b). The PCoA vertical axis of eukaryotes explained 9.4% of the distribution variation observed, while the horizontal axis determined 42.1% of the variability (Figure 3.5c).

The relative abundance of bacteria, fungi and eukaryotes are shown in the Figure 3.6. The obtained taxonomy annotation of the samples showed that *Proteobacteria* and

Bacteroidetes were the most abundant bacterial phylum in the conventional and organic samples (Figure 3.6a). However, the relative abundance of the *Proteobacteria* was higher in the conventional soil samples, while *Bacteroidetes* was higher in the organic soil samples. However, little effects of biostimulant treatments were observed. The relative abundance of the fungi showed that *Sordariomycetes* was one of the most abundant fungus class in conventional and organic soil samples. In addition, the abundance of *Mortierellomycetes* in organic soil samples was higher than conventional. In contrast, *Eurotiomycetes* appeared to be the most abundant class in the conventional soil samples (Figure 3.6b). Besides, the eukaryote relative abundance results showed that fungi and *Metazoa* were the most abundant orders in the conventional and organic soil samples, respectively (Figure 3.6c). The relative abundance of fungi was higher for the conventional soil samples, while *Metazoa* was higher for the organic soil samples.

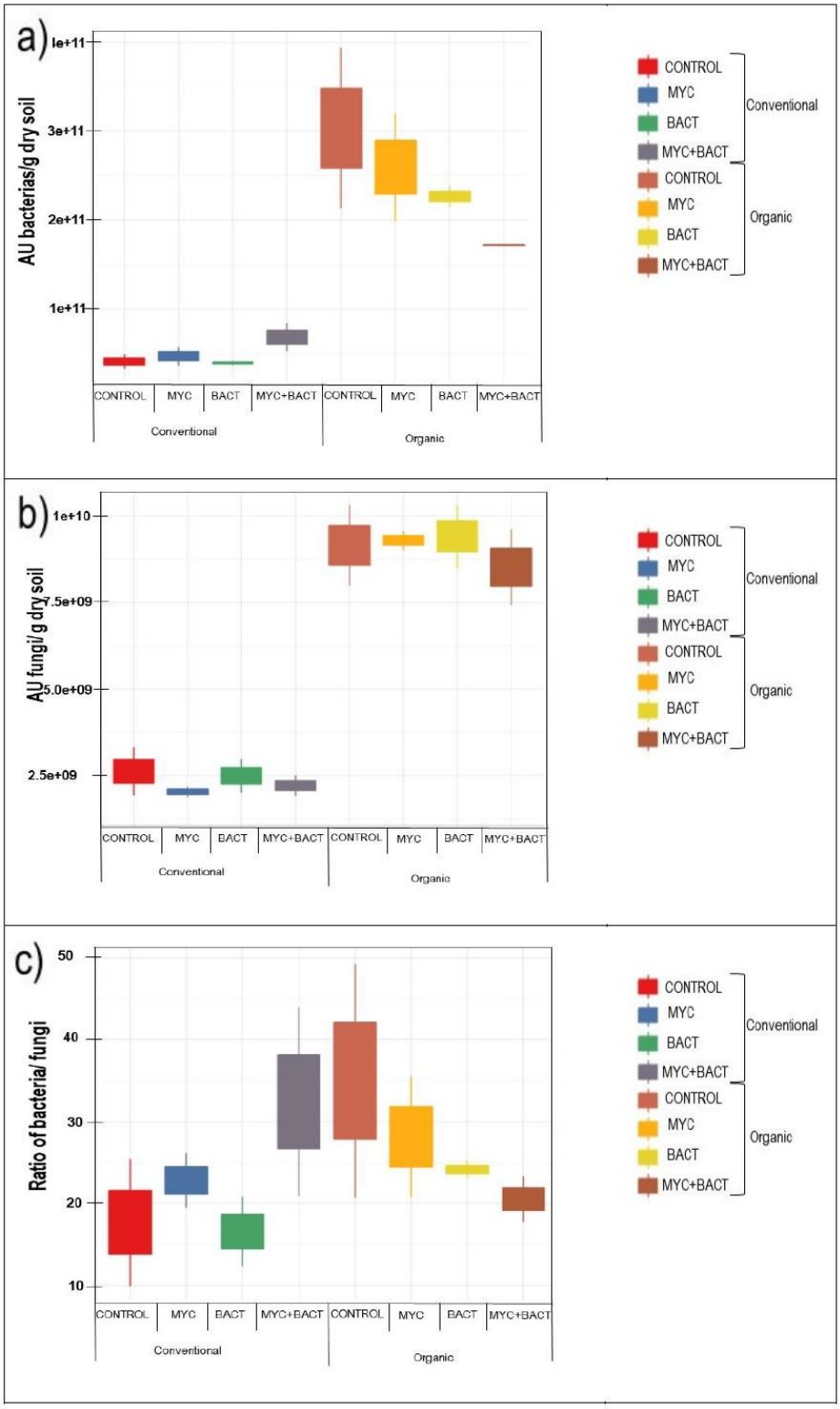

Figure 3.3 Soil quantification of a) bacteria, b) fungi, and c) bacteria/ fungi in the treatments (n=2) of conventional and organic crops.

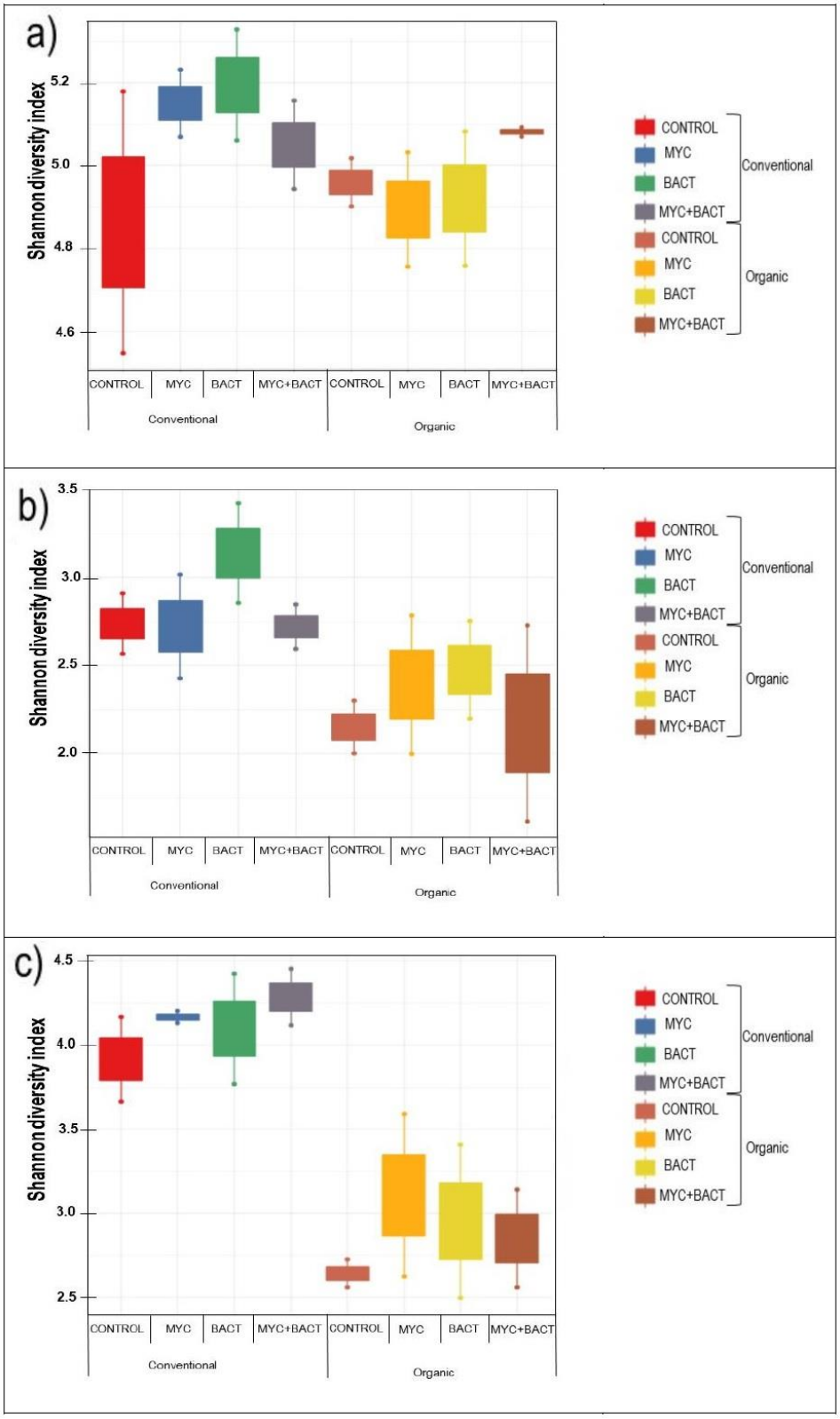

Figure 3.4 Dominant a) bacteria, b) fungi, and c) eucaryotic groups expressed by Shannon's index in the growing medium for each treatment (n=2) of conventional and organic crops.

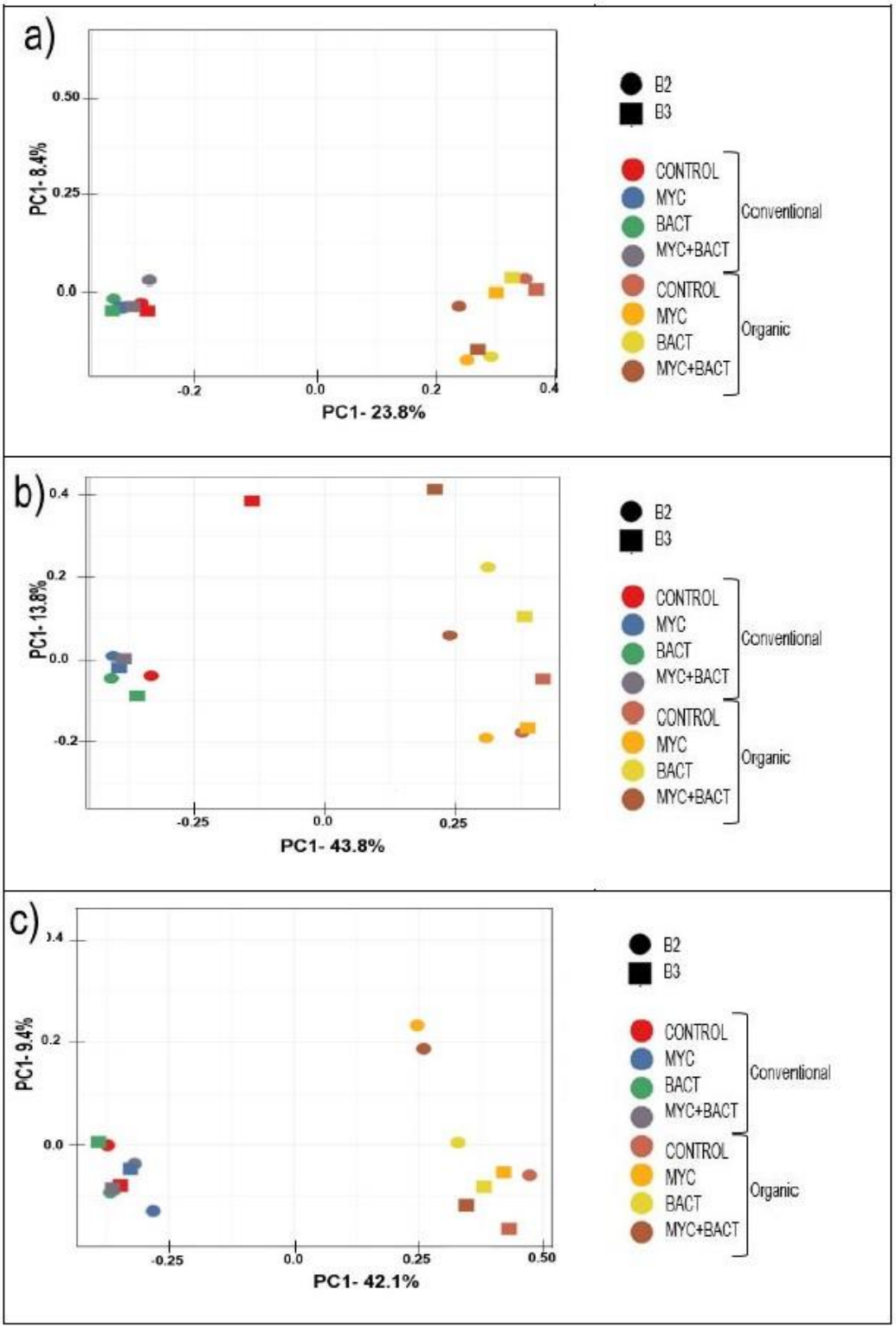

Figure 3.5 PCoA of a) bacteria, b) fungi, and c) eukaryote growing medium communities for conventional and organic soil samples during the greenhouse experiment (n=2).

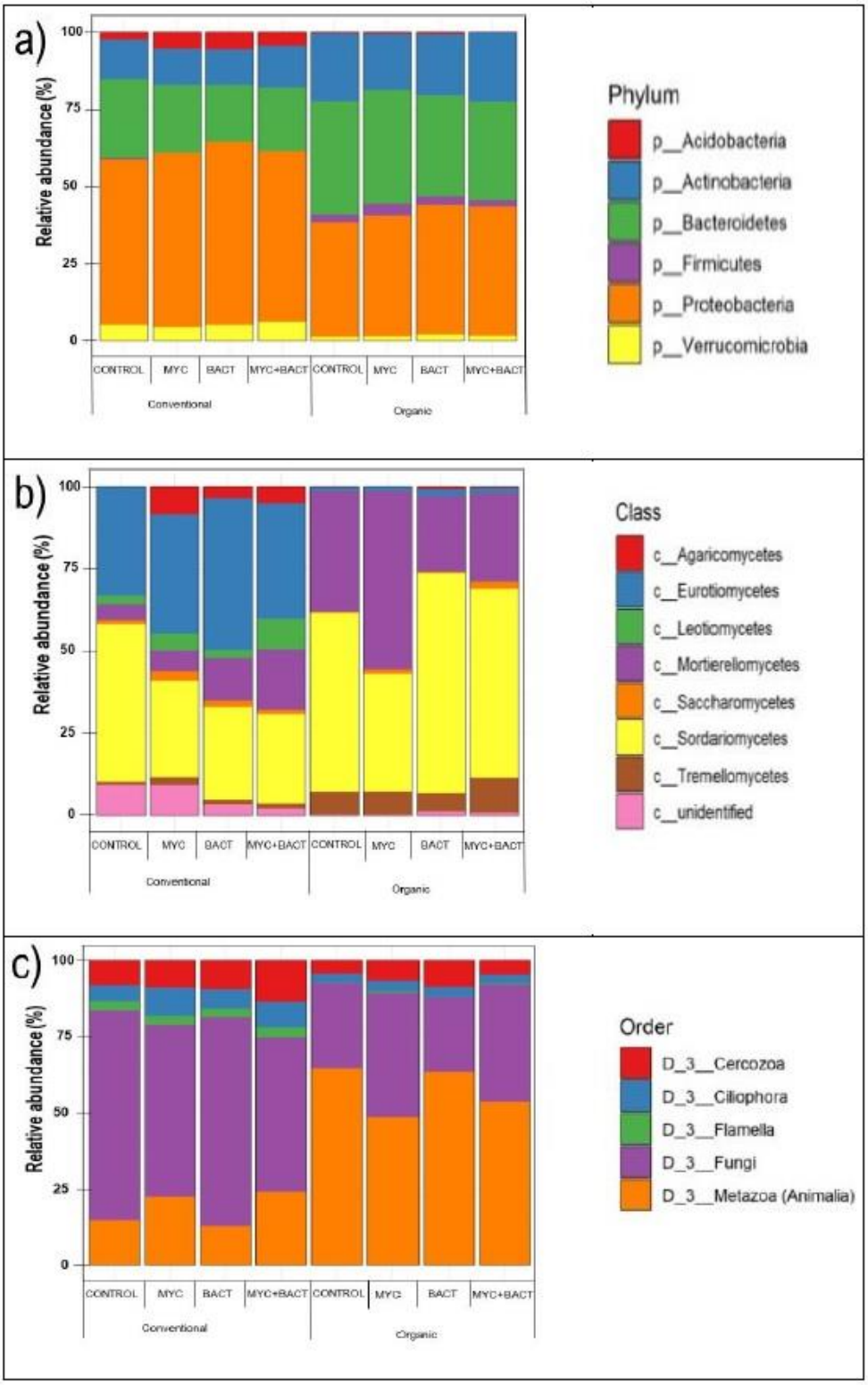

Figure 3.6 The relative abundance of the principal groups of a) bacteria, b) fungi, and c) eukaryote in the growing medium of conventional and organic crops after q PCR analysis during the greenhouse experiment (n=2).

3.1.1.3 CO_2 efflux of growing medium

The CO_2 efflux of the growing media was significantly different (P<0.001) between treatments (Figure 3.7). For both growing systems, biostimulants increased the CO_2 efflux compared to the corresponding controls, except for the organic treatments under a low fertilization and citric acid applications. Regardless the biostimulant treatments, values of CO_2 efflux were higher (+220%) for organic cultivation compared with conventional (Figure 3.8).

Specifically, the highest value of CO_2 efflux in conventional and organic growing systems was observed in the treatment with mycorrhiza (MYC) compared to the control (+154% and +137%, respectively) (Figure 3.7). Besides, in both growing systems, treatments with seaweed extract (SEAWEED), a combination of bacteria (BACT), and a mixture of mycorrhiza and bacteria (MYC+BACT) increased in average CO_2 efflux compared to the control (+44%, +46%, and +51%, respectively). In addition, *Trichoderma* increased the soil CO_2 efflux under conventional cultivation (128%).

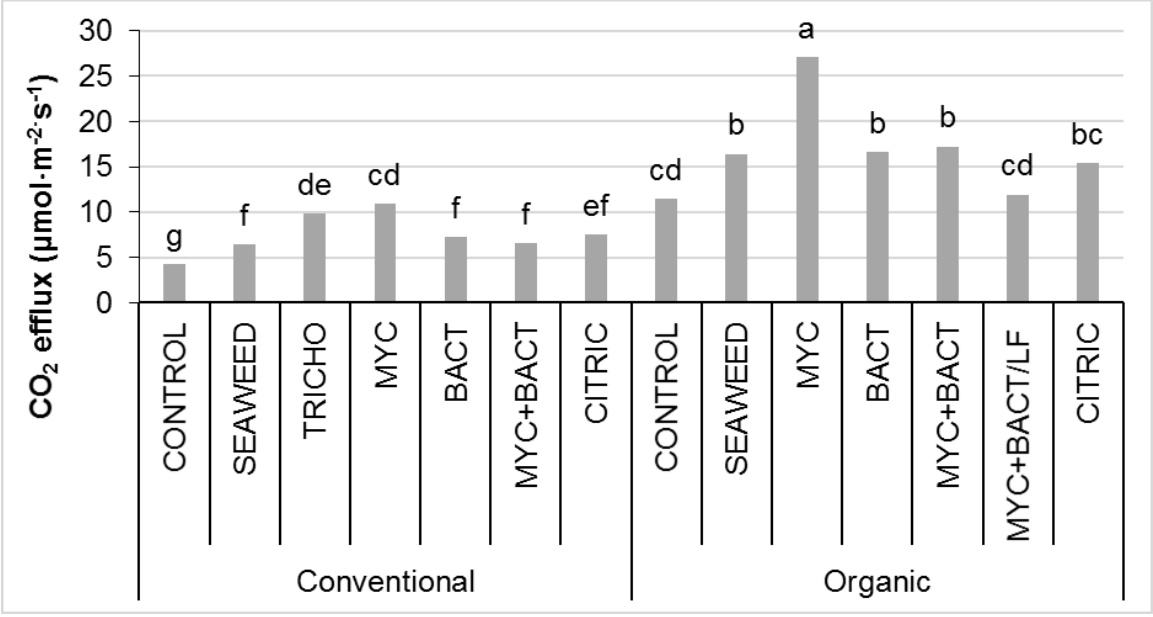

Figure 3.7 Substrate CO2 efflux of strawberry plants treated with studied biostimulants. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5) (See annex B.1 for data and P values).

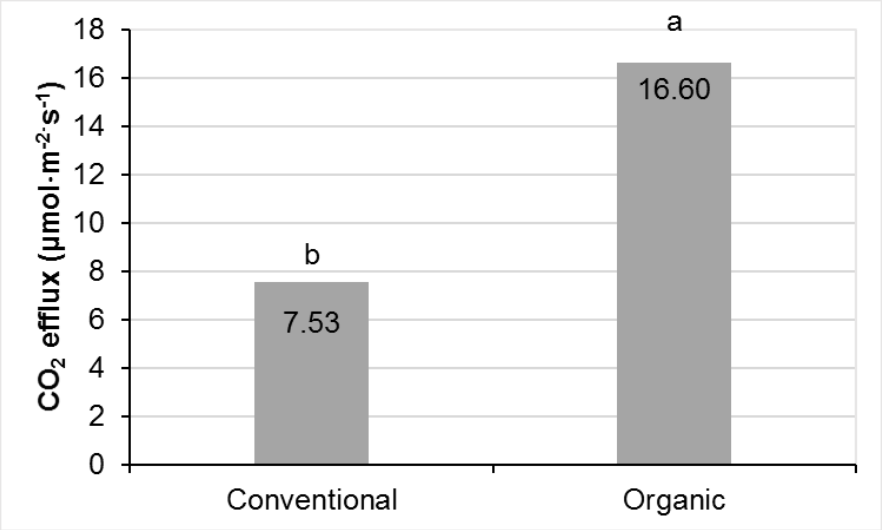

Figure 3.8 Variations of substrate CO_2 efflux of strawberry plants by growing system during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05). Substrate CO_2 efflux expressed by µmol·m^{-2}·s^{-1} (See annex B.1 for data and P values).

3.1.2 Leaf photosynthetic performance

3.1.2.1 Leaf chlorophyll fluorescence

The chlorophyll fluorescence parameters are shown in Table 3.1. Results showed no significant differences between treatments for the maximum quantum efficiency of photosystem II expressed by Fv/Fm. However, Fv/ Fm was lower in April compared to the other measurements performed in March and May (Table 3.1). There was no interaction between studied treatments and time for the fluorescence parameters.

However, significant differences between biostimulant treatments (P<0.001), time of measurement (P<0.001) and crop systems (P<0.05) were observed for the performance index (P Index) of strawberry plants. For both growing systems, biostimulants did not significantly influence the performance index of strawberry leaves compared with their respective control. However, under conventional management, treatment with *Trichoderma* (TRICHO) increased P Index by 17% compared to seaweed extract (SEAWEED), mycorrhiza (MYC), and citric acid (CITRIC).

Seaweed extracts and citric acid under organic farming induced a higher value of P Index compared to the combination of mycorrhiza and bacteria under low fertilization (MYC+BACT/LF). Besides, the highest value for P Index was observed in May compared to the other measurement dates. Regardless of the time of measurements and biostimulant treatment, plants grown organically had a 5% higher P Index compared to conventionally grown plants.

3.1.2.2 Leaf chlorophyll content

There was a difference between treatments (P<0.001) and measurement time (P<0.001) for leaf chlorophyll content, while no interaction between treatments and time was observed (Table 3.1). However, the leaf chlorophyll content of conventionally and organically grown plants was similar.

Under organic farming, the use of bacteria decreased leaf chlorophyll content compared with its respective control and citric acid treatment, while the use of a low fertilization significantly reduced leaf chlorophyll content compared to the other treatments. On the other hand, the leaf chlorophyll content of plants treated with mycorrhiza under organic cultivation was higher than same treatment under conventional one. Regardless of the treatments, leaf chlorophyll content increased from March to May.

Table 3.1 Chlorophyll fluorescence parameters (Fv/Fm and P Index) and chlorophyll content of strawberry leaves cultivated in greenhouse under different combination of growing systems and biostimulants (n=45).

Treatments		**Fv/Fm**	**P Index**	**chlorophyll content (SPAD unit)**
Conventional	**CONTROL**[z]	0.800	2.78 abc[x]	37.5 ab
	SEAWEED	0.803	2.67 bc	36.9 ab
	TRICHO	0.803	3.07 a	37.2 ab
	MYC	0.798	2.56 cd	36.7 b
	BACT	0.800	2.63 bc	37.2 ab
	MYC+BACT	0.799	2.70 abc	37.3 ab
	CITRIC	0.801	2.66 cd	36.9 ab
Organic	**CONTROL**	0.804	2.95 ab	38.1 a
	SEAWEED	0.806	3.08 a	37.4 ab
	MYC	0.802	2.85 abc	38.1 a
	BACT	0.800	2.83 abc	37.7 b
	MYC+BACT	0.804	2.97 ab	37.2 ab
	MYC+BACT/LF	0.801	2.25 d*	34.4 c*
	CITRIC	0.807	3.09 a	38.1 a
Time	March	0.807a	2.39 b	36.0 b
	April	0.789b	2.37 b	36.5 b
	May	0.810a	3.60 a	39.0 a
Growing systems	Conventional	0.80	2.72 b	37.10
	Organic	0.80	2.86 a	37.29
P values				
Biostimulant (B)		0.375	<0.001	<0.001
Time (T)		<0.001	<0.001	<0.001
B × T		0.994	0.960	0.833
Conventional vs Organic		0.172	0.044	0.388

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO= *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).

[x]means of the same column with different letters are significantly different at $P<0.05$.

*Treatments are different from their respective control.

3.1.2.3 Photosynthesis light saturation curves

The light saturation curves related to each treatment is shown in Figure 3.9. For leaves grown under conventional management, biostimulant treatments had no positive effects on leaf photosynthetic rate under low PPFD. On the other hand, at higher PPFD of 500

μmol $CO_2 m^{-2} s^{-1}$, biostimulants increased their photosynthetic rate compared with control plants (Figure 3.9). Under organic management lower photosynthetic rate was observed under moderate and high PPFD for the mycorrhiza (MYCO) and citric acid (CITRIC) treatments, while higher photosynthetic rate was observed for bacteria (BACT) and the combination of mycorrhiza and bacteria under low fertilization (MYC+BACT/LF) treatments compared with control. However, no significant difference was observed between treatments for the maximum rate of photosynthesis, maximum quantum yield, and dark respiration rate (Table 3.2). However, maximum rate of photosynthesis was 11.37% higher for conventionally grown plants compared with organically-grown plants.

Table 3.2 Photosynthesis parameters of strawberry leaves cultivated in greenhouse under different combination of growing systems and biostimulants (n=5).

Treatments		**Maximum rate of photosynthesis (μmol CO_2 m^{-2} s^{-1})**	**Respiration rate in the dark (Rd) (μmol CO_2 m^{-2} s^{-1})**	**Maximum quantum yield (Φ)**
Conventional	**CONTROL**[z]	17.24	-0.365	0.068
	SEAWEED	17.94	-0.835	0.076
	TRICHO	19.64	-0.609	0.073
	MYC	18.98	-0.734	0.078
	BACT	18.21	-0.919	0.075
	MYC+BACT	18.84	-0.241	0.068
	CITRIC	17.42	-0.752	0.077
Organic	**CONTROL**	16.26	-0.441	0.072
	SEAWEED	16.96	-0.531	0.075
	MYC	16.00	-0.641	0.070
	BACT	16.91	-0.424	0.071
	MYC+BACT	15.67	-0.312	0.069
	MYC+BACT/ LF	18.57	-0.745	0.081
	CITRIC	14.80	-0.741	0.077
Growing systems	Conventional	18.32a	-0.636	0.074
	Organic	16.45b	-0.548	0.074
P values				
Biostimulant (B)		0.267	0.308	0.399
Conventional vs Organic		0.008	0.387	0.996

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO= *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).

[x]means of the same column with different letters are significantly different at $P<0.05$.

*Treatments are different from their respective control.

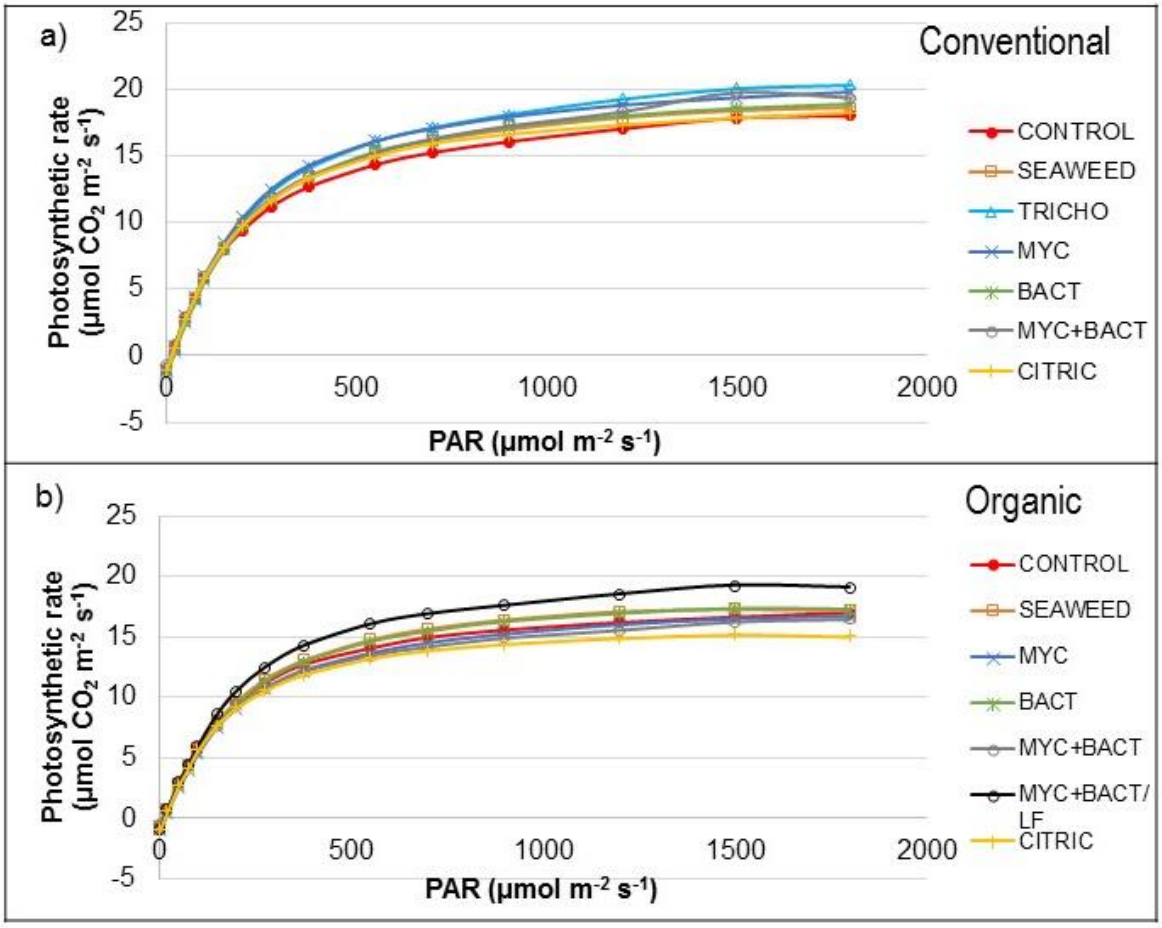

Figure 3.9 Light saturation curves of strawberry leaves cultivated in greenhouse under different combinations of growing systems and biostimulants (n=5).

3.1.3 Non-destructive growth parameters

Our results showed that growth parameters such as the number of leaves, number of flowering stalks, number of crowns, and diameter of crowns were influenced by the application of biostimulants and time of measurement (Table 3.3). However, in general, biostimulant treatments did not improve plant growth compared with control treatments. We observed an interaction between treatments and time of measurement for the number of leaves, the number of flowering stalks, and the diameter of crowns ($P \leq 0.05$). The interaction between treatments and time for both growing systems are presented in Figures 3.10, 3.11, and 3.12. Specifically, in conventional and organic grown plants, the number of leaves was not significantly influenced by biostimulants except in May, where

seaweed extract decreased the number of leaves compared with conventional control, *Trichoderma*, and citric acid treatments (Figure 3.10). The lower fertilization of organically-grown plants reduced the number of leaves in April and May.

Regarding the growing system, the number of leaves under conventional cultivation was slightly higher than under organic cultivation (P=0.025) (Table 3.3). As expected, regardless of the treatments, plant growth parameters increased during the experiment, as shown by the significant differences between March, April, and May.
Similar results were observed for the number of flowering stalks (Figure 3.11). In March and April, no significant difference was observed between biostimulants compared with control treatments. In May, conventionally grown plants with a mixture of mycorrhiza and bacteria (MYC+BACT) produced a lower number of flowering stalks compared to the control (-23%). Plants treated with citric acid had a higher number of flowering stalks compared with seaweed, *Trichoderma*, bacteria and the mixture of mycorrhiza and bacteria treatments. Under organic management, plants treated with citric acid had a higher number of flowering stalks compared with seaweed and mycorrhiza treatments. The lower fertilization treatment (MYC+BACT/LF) reduced the number of flowering stalks (-25%) compared with organic control treatment.

The diameter of crowns was significantly lower in March for conventional plants treated with a mixture of mycorrhiza and bacteria (MYC+BACT; -26%) compared to its control (Figure 3.12a). Under organic cultivation, plants treated with seaweed extract and bacteria had a lower crown diameter in March than their control plants (Figure 3.12b). In April and May, however, no difference between treatments was observed, except for the organic seaweed treatment in April and the low fertilization treatment (MYC+BACT/LF) in April and May.

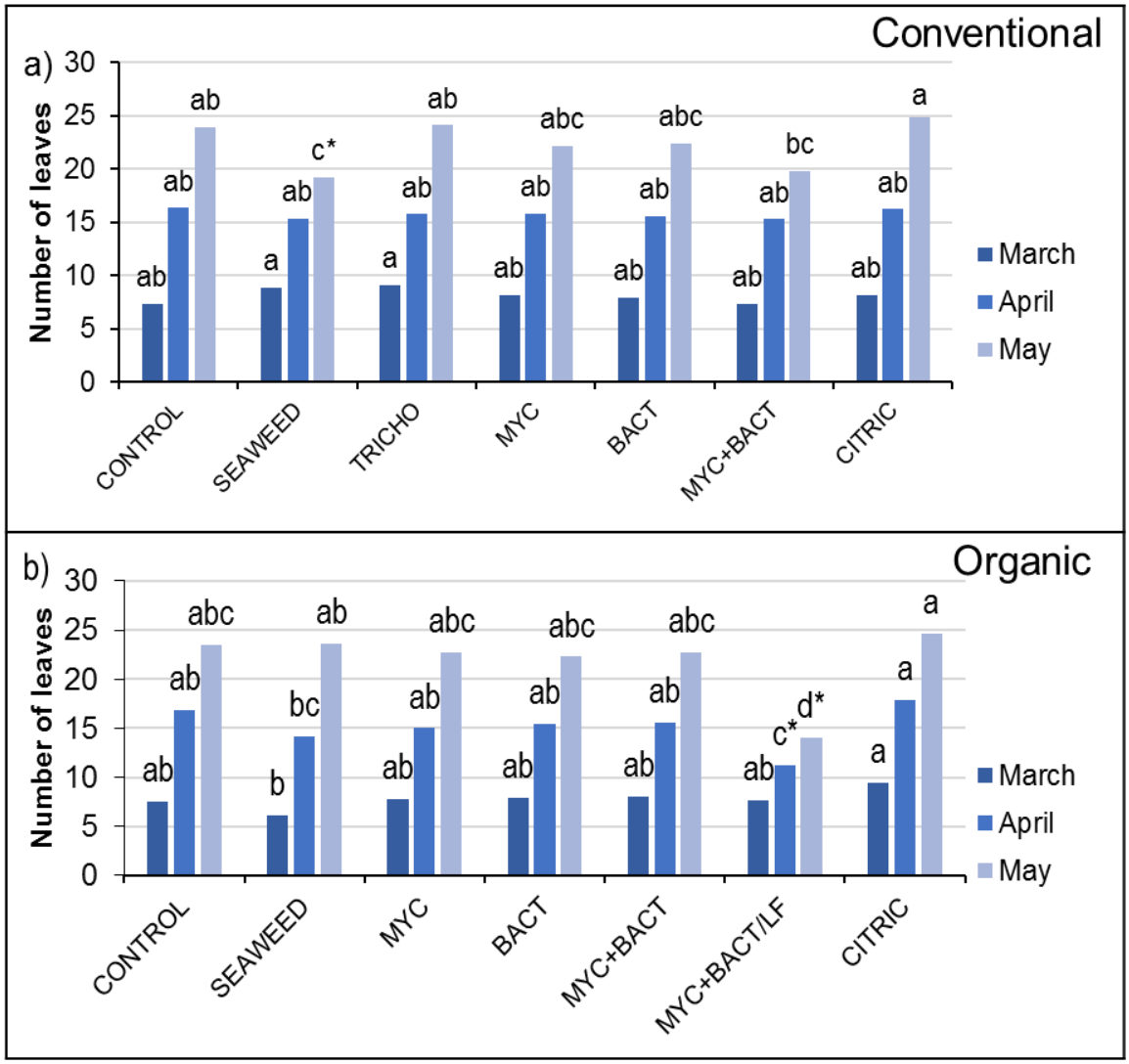

Figure 3.10 The Influence of time on the number of leaves in strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

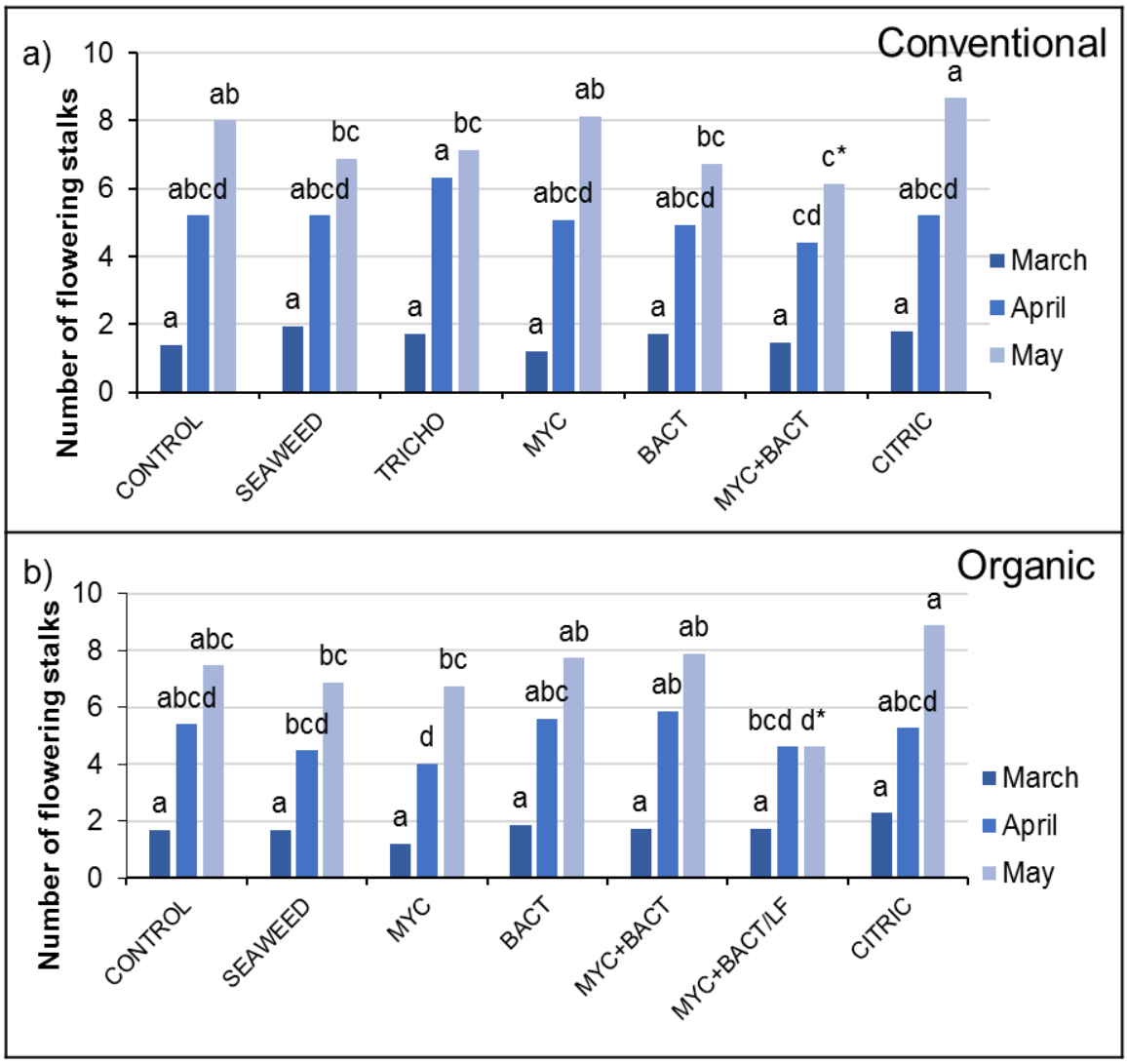

Figure 3.11 The influence of time on the number of flowering stalks in strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

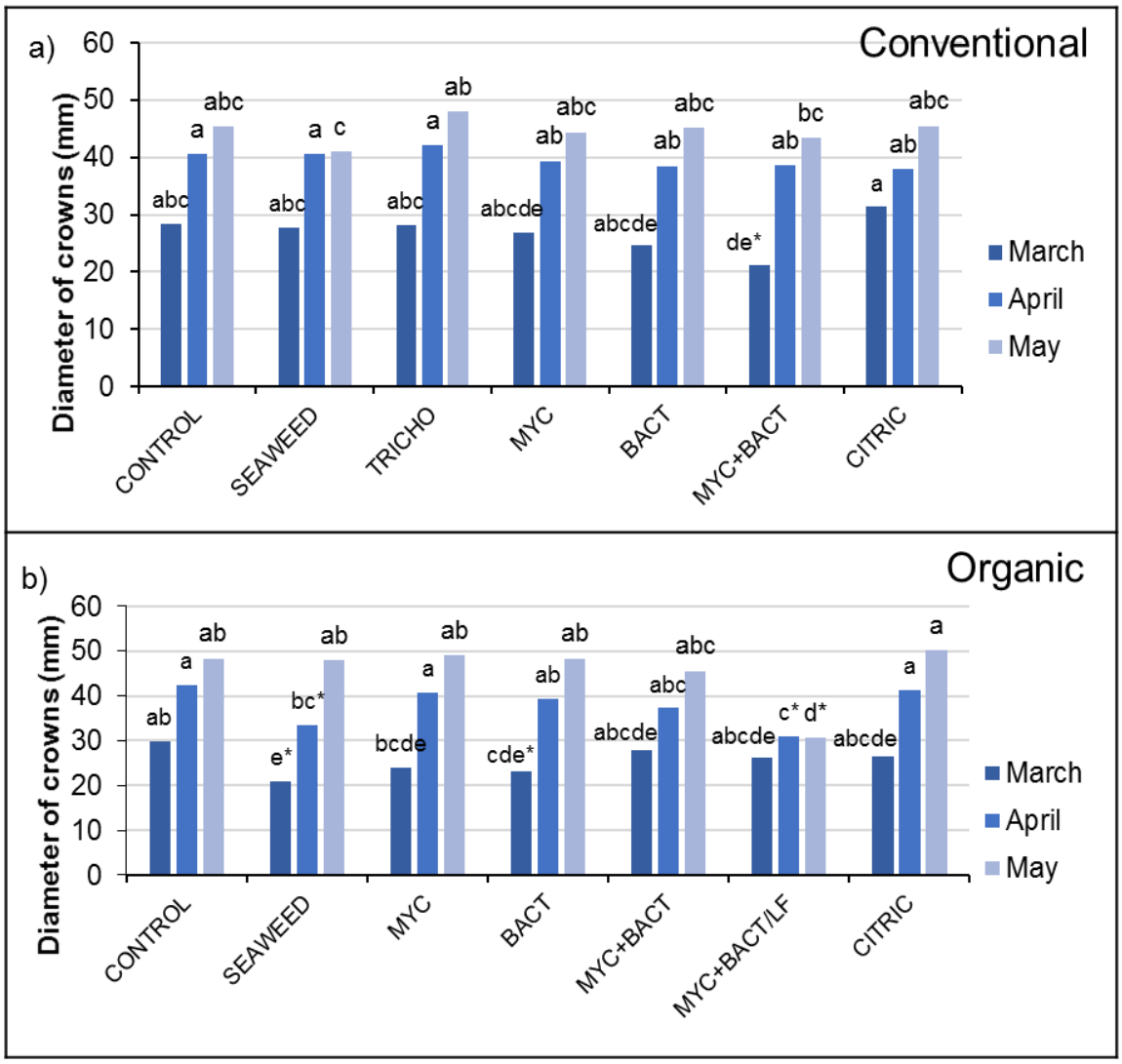

Figure 3.12 The influence of time on the diameter of crowns in strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

Table 3.3 Growth parameters of strawberry plants cultivated in greenhouse under different combination of growing systems and biostimulants (n=45).

Treatments		Number of leaves	Number of flowering stalks	Number of crowns	Diameter of crowns (mm)
Conventional	**CONTROL**[z]	15.87 abc	4.87 abc[x]	3.53 abc	38.28 ac
	SEAWEED	14.47 bc	4.67 abcde	3.20 bc	37.03 abcd
	TRICHO	16.31 ab	5.07 abc	3.49 abc	39.41 ab
	MYC	15.36 abc	4.80 abcd	3.33 abc	36.99 abcd
	BACT	15.31 abc	4.47 bcde	3.33 abc	36.06 bcd
	MYC+BACT	14.13 c	4.00 def*	3.24 abc	34.35 cd
	CITRIC	16.40 ab	5.22 ab	3.67 a	38.33 abc
Organic	**CONTROL**	15.93 abc	4.84 abc	3.42 abc	40.24 a
	SEAWEED	14.67 bc	4.33 cdef	3.36 abc	33.40 d
	MYC	15.18 bc	3.98 ef	3.18 c	37.62 abcd
	BACT	15.20 bc	5.07 abc	3.31 abc	36.58 abcd
	MYC+BACT	15.47 abc	5.16 ab	3.42 abc	36.79 abcd
	MYC+BACT/LF	10.98 d*	3.64 f*	2.64 d*	30.19 e*
	CITRIC	17.31 a	5.47 a	3.64 ab	39.04 ab
Time	March	7.96 c	1.67 c	2.31 c	26.21 c
	April	15.46 b	5.11 b	3.53 b	38.79 b
	May	22.13 a	7.27 a	4.18 a	45.22 a
Growing systems	Conventional	15.41 a	4.73	3.40	37.21
	Organic	14.96 b	4.64	3.28	36.27
P values					
Biostimulant (B)		<0.001	<0.001	0.005	<0.001
Time (T)		<0.001	<0.001	<0.001	<0.001
B × T		0.048	0.035	0.178	0.017
Conventional vs Organic		0.025	0.585	0.178	0.350

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).

[x]means of the same column with different letters are significantly different at P<0.05.

*Treatments are different from their respective control.

3.1.4 Destructive growth parameters

3.1.4.1 Fresh and dry biomass

The results of the shoot and root fresh and dry biomass are present in Table 3.4. All parameters were significantly influenced by the biostimulant treatments (P<0.001) and the growing system (P<0.001).

For conventionally-grown plants, seaweed extract decreased dry shoot biomass (-26%), as well as fresh and dry root biomass (-40%) compared with its control, while citric acid decreased fresh (-25%) and dry (-30%) root biomass. On the other hand, under organic management, citric acid increased fresh (+32%) and dry (+35%) root biomass compared with its respective control. Plants treated with a mixture of mycorrhiza and bacteria with low fertilization (MYC+BACT/LF) had, however, a lower fresh and dry shoot biomass compared to the control (-50%), while no statistical difference between biostimulants and the control was observed.

Regardless of the biostimulant treatments, conventionally-grown plants had the highest value of fresh (+24%) and dry (+24%) shoot biomass, while fresh and dry root biomass were 48% and 54% higher, respectively, compared to the organically-grown plants.

3.1.4.2 Leaf area

A significant difference was observed between biostimulant treatments and growing systems (Table 3.4). Under conventional cultivation, control, mycorrhiza and the mixture of mycorrhiza and bacteria recorded the highest leaf area, while the lowest leaf area was observed for the seaweed extract (-26%). Biostimulants did not impact leaf area compared with control treatments, except for seaweed extract that reduced leaf area (-21%) of conventionally-grown plants. In contrast, under organic cultivation, leaf area of seaweed treatment was higher than mycorrhiza, the mixture of mycorrhiza and bacteria and the mixture of mycorrhiza and bacteria with low fertilization, but not significantly different of the control and citric acid treatments. However, leaf area of conventional grown plants was 20% higher than organic grown plants, regardless the biostimulant treatments.

Table 3.4 Fresh and dry biomass of strawberry plants cultivated in greenhouse under different combinations of biostimulants and growing systems (n=15).

Treatments		Shoot fresh biomass (g)	Shoot dry biomass (g)	Root fresh biomass (g)	Root dry biomass (g)	Leaf area (cm^2 $plant^{-1}$)
Conventional	**CONTROL**[z]	147.54 abc[x]	35.30 ab	86.13 a	12.97 a	2750 a
	SEAWEED	121.04 cde	26.19 d*	51.38 cd*	7.90 cdef*	2184 bc*
	TRICHO	143.65 abcd	32.11 abcd	68.19 ab	10.05 abc	2605 ab
	MYC	154.73 ab	35.45 ab	74.57 ab	10.84 ab	2736 a
	BACT	144.51 abc	34.25 abc	67.97 ab	10.16 abc	2621 ab
	MYC+BACT	166.68 a	36.34 a	71.98 ab	10.82 ab	2776 a
	CITRIC	140.41 abcd	32.28 abcd	64.70 bc*	9.10 bcde*	2565 ab
Organic	**CONTROL**	125.44 cde	29.70 abcd	46.20 de	6.72 fg	2347 abc
	SEAWEED	136.66 bcde	29.54 bcd	46.99 cde	6.62 efg	2771 a
	MYC	116.34 de	26.93 d	43.11 de	6.39 fg	2179 bc
	BACT	120.65 cde	27.93 cd	42.36 de	5.99 fg	2343 abc
	MYC+BACT	111.10 e	26.13 d	49.94 cde	6.98 def	2055 c
	MYC+BACT/LF	62.902 f*	14.99 e*	38.06 e	4.98 g	1172 d*
	CITRIC	147.83 abc	32.45 abcd	60.99 bc*	9.04 bcd*	2492 abc
Growing systems	Conventional	145.51 a	33.13 a	69.27 a	10.26 a	2605 a
	Organic	117.27 b	26.81 b	46.81 b	6.67 b	2169 b
P values						
Biostimulant (B)		<0.001	<0.001	<0.001	<0.001	<0.001
Conventional vs Organic		<0.001	<0.001	<0.001	<0.001	<0.001

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).
[x]means of the same column with different letters are significantly different at P<0.05.
*Treatments are different from their respective control.

3.1.5 Foliar mineral content

Table 3.5 shows that studied biostimulant treatments (P<0.0001) and sampling time (P<0.0001) had a significant effect on nitrogen (N), phosphorous (P), potassium (K), and calcium (Ca) leaf concentration. However, we observed a significant biostimulants interaction by time for P, K and Ca concentrations (P= 0.012) (Table 3.6). There was also a significant difference between conventional and organic growing systems for N, P and Ca leaf concentrations.

Nitrogen- The lowest value for N concentration was observed in the treatment with low fertilization (MYC+BACT/LF) (Table 3.5). Citric acid significantly decreased (-10%) the concentration of the nitrogen in the leaf of organically-grown plants compared with its control. The other biostimulant treatments did not affect the N concentration of the leaf compared with their respective control. Leaves of organically-grown plants had 5.7% higher N content compared with leaves of conventionally-grown plants. Regardless of the treatments, leaf N concentration was 36% higher for leaves sampled in March compared with leaves sampled in May (Table 3.6).

Phosphorus- Biostimulant treatments did not significantly affect the leaf P concentration of conventionally and organically grown plants compared with their respective control (Table 3.6). However, the low fertilization treatment (MYC+BACT/LF) had a lower leaf P concentration in May (-36%) compared with its control. Regardless of the biostimulant treatments, conventionally-grown plants had higher P concentration (+27%) than organically-grown plants. In general, leaves collected in March had 41% more P concentration than leaves collected in May.

Potassium- Similarly to the leaf P concentration, biostimulant treatments did not significantly affect the leaf K concentration of plants compared to their respective control. However, organically-grown plants under low fertilization (MYC+BACT/LF) had lower leaf K concentration in March (-13%) and May (-27%) compared with the control. In general, leaves samples collected in March had higher (13%) leaf K concentration than leaves collected in May. No significant difference was observed between both growing systems.

Calcium- In March, *Trichoderma* and citric acid treatments increased by 38% and 53% the leaf Ca concentration of conventionally-grown plants compared with its control, while no effect was observed in May. Regardless of the biostimulant treatments, the leaf Ca

concentration of organically-grown plants were 15% lower than the conventionally-grown plants. In general, leaves sampled in March had 4% higher Ca concentration than leaves sampled in May.

Magnesium- For leaf Mg concentration, there was no significant difference between treatments, although leaves sampled in March had 35% more Mg than leaves collected in May. No significant difference was observed between both growing systems.

Table 3.5 Leaf mineral concentrations in the percentage of leaf dry weight of strawberry plants cultivated in greenhouse under different combinations of biostimulants and growing systems (n=12).

Treatments		N	$N\text{-}NO_3$	$N\text{-}NH_4$	P	K	Ca	Mg
Conventional	**CONTROL**[z]	1.83 e[x]	125.33 abc	4.53 c	0.466 a	1.41 ab	0.63 abc	0.28
	SEAWEED	1.93 de	83.37 c	5.50 bc	0.461 ab	1.37 ab	0.66 abc	0.29
	TRICHO	1.98 bcde	109.30 abc	3.00 c	0.485 a	1.42 ab	0.67 abc	0.31
	MYC	2.02 abcde	97.53 abc	5.60 bc	0.477 a	1.40 ab	0.72ab	0.31
	BACT	1.89 e	71.77 c	5.67 bc	0.471 a	1.32 b	0.68 abc	0.29
	MYC+BACT	1.95 de	87.77 bc	5.73 bc	0.453 abc	1.35 ab	0.58 bcd	0.27
	CITRIC	1.86 e	74.03 c	5.07 bc	0.494 a	1.42 ab	0.78 a	0.33
Organic	**CONTROL**	2.19 ab	147.27 abc	8.00 bc	0.399 bcd	1.44 a	0.54 cd	0.31
	SEAWEED	2.15 abcd	156.30 abc	7.33 bc	0.393 cd	1.46 a	0.46 d	0.30
	MYC	2.00 abcde	207.00 a	30.93 a	0.371 de	1.38 ab	0.53 cd	0.30
	BACT	2.21 a	220.33 ab	22.43 a	0.387 d	1.37 ab	0.61 bc	0.33
	MYC+BACT	2.18 abc	195.00 a	19.80 ab	0.372 d	1.39 ab	0.60 bcd	0.34
	MYC+BACT/LF	1.55 f*	14.30 d	8.70 bc	0.304 e*	1.14 c*	0.64 abc	0.29
	CITRIC	1.96 cde*	138.23 abc	27.03 a	0.394 cd	1.43 ab	0.59 bcd	0.31
Growing systems	Conventional	1.92	92.73	5.01 b	0.47 a	1.38	0.67 a	0.28
	Organic	2.03	154.06	17.75 a	0.37 b	1.37	0.57 b	0.29
Time	March	2.28 a			0.734 a	2.16 a	0.94 a	0.35 a
	May	1.67 b			0.522 b	1.91 b	0.90 b	0.26 b
P values								
Biostimulant (B)		<.0001	<.0001	0.006	<.0001	0.001	0.015	0.235
Time (T)		<.0001			<.0001	<.0001	<.0001	<.0001
B × T		0.160			<.0001	<.0001	0.012	0.429
Conventional vs Organic		0.012	0.200	<.0001	<0.001	0.609	<0.001	0.150

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).
[x]means of the same column with different letters are significantly different at P<0.05.
*Treatments are different from their respective control.

Table 3.6 Interaction effect between biostimulants and measurement time for leaf mineral concentrations of strawberry plants.

Treatments		Phosphorus (%)		Potassium (%)		Calcium (%)	
		March	May	March	May	March	May
Conventional	**CONTROL**[z]	0.784 abcd	0.592 abc	2.24 abc	1.91 bcd	0.836 cd	1.034 abc
	SEAWEED	0.816 abc	0.546 abcd	2.15 abcd	1.89 cde	1.042 abc	0.922 abcd
	TRICHO	0.884 a	0.554 abcd	2.33 a	1.88 cde	1.154 ab*	0.832 bcd
	MYC	0.784 abcd	0.632 a	2.31 ab	1.85 de	1.082 abc	1.068 ab
	BACT	0.766 abcd	0.624 ab	2.07 cde	1.85 de	0.934 bcd	1.068 ab
	MYC+BACT	0.828 ab	0.514 bcd	2.26 abc	1.74 e*	0.908 bcd	0.8 bcd
	CITRIC	0.890 a	0.574 abc	2.27 abc	1.93 abcde	1.28 a*	1.048 abc
Organic	**CONTROL**	0.694 bcde	0.494 cd	2.19 abcd	2.06 abc	0.828 cd	0.778 bcd
	SEAWEED	0.654 cde	0.516 bcd	2.20 abcd	2.11 ab	0.692 d	0.658 d
	MYC	0.652 cde	0.450 d	2.00 de	2.04 abcd	0.864 bcd	0.706 cd
	BACT	0.666 bcde	0.484 cd	2.09 bcde	1.92 abcde	0.972 abcd	0.836 bcd
	MYC+BACT	0.616 de	0.490 cd	2.12 abcde	1.98 abcd	0.958 bcd	0.802 bcd
	MYC+BACT/LF	0.590 e	0.316 e*	1.91 e*	1.51 f*	0.706 d	0.688d
	CITRIC	0.650 cde	0.522 abcd	2.09 bcde	2.12 a	0.874 bcd	0.884 abcd

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).
[x]means of the same column with different letters are significantly different at P<0.05.
*Treatments are different from their respective control.

3.1.6 Yield

The yield parameters evaluated during the greenhouse experiment were the total yield, marketable yield, and unmarketable yield. Significant effects of biostimulants were observed for all yield parameters, although significant interactions were observed between biostimulants and time, except for the number of total and marketable fruits were no significant interaction was observed (Table 3.7).

3.1.6.1 Total yield (marketable and unmarketable fruits)

The total number of fruits per plant per week showed a significant difference between treatments (P<0.001) and the time of harvest (P<0.001). Besides, a significant difference was observed between organic and conventional growing systems (P<0.001). Strawberry plants with foliar application of citric acid (CITRIC) in the conventional growing system produced a higher number of total and marketable fruits (+20% on average) compared with its respective control, while the mixture of mycorrhiza and bacteria (MYC+BACT) produced a lower number of total fruits (-12%). Similarly, for the organic growing system, though not significant different, treated plants with citric acid had 15% and 11% more total and marketable fruit number compared with the organic control. However, the total and marketable number of fruits were lower (-46 and -44%, respectively) in the treatment with low fertilization (MYC+BACT/LF) compared with the organic control.

Like fruit number, a significant difference between biostimulants (P<0.001), time of harvest (P<0.001), and organic and conventional growing systems (P<0.001) was observed for the total weight of fruits per plant (total yield). However, a significant interaction occurred between biostimulants and the time of harvest (P=0.004; Figure 3.13). For both growing systems, the influence of the biostimulants on the total yield appeared at weeks nine, 16, and 17 after plantation. In the conventional system, plants treated with a combination of bacteria (BACT) produced a higher yield at the third and tenth harvests (weeks 9 and 16) compared with the control (P=0.046) (Fig. 3.13a). At the last date of harvest (17 weeks after plantation), however, the highest yield was observed for the plants treated with a mixture of mycorrhiza and bacteria (MYC+BACT; P=0.008), followed by *Trichoderma* (TRICHO; P=0.026) and seaweed (SEAWEED; P=0.066).

For the organically-grown plants, citric acid (CITRIC; P=0.017) significantly increased the total yield (+53%) at the third harvest (nine weeks after plantation) compared to the control,

while the lowest total yield was observed for plants treated with low fertilization (MYC+BACT/LF) at 16 (-54%) and 17 (-49%) weeks after plantation compared with the other treatments. For the other harvesting weeks, no significant difference between treatments was observed. On the other hand, when the seasonal total yield is considered, regardless of the harvest weeks, plants sprayed with citric acid had higher total fruit weight per plant compared to the control in both growing systems (+16% conv. and +9% org.), although this influence was only significant for the conventional cultivation. Like the total number of fruits, plants grown under low fertilization (MYC+BACT/LF) had the lowest total weight of fruits (-38%) compared to the organic control.

Total yield (total number and weight of fruits) was 16% lower for organically-grown plants than for conventionally-grown plants (Table 3.7).

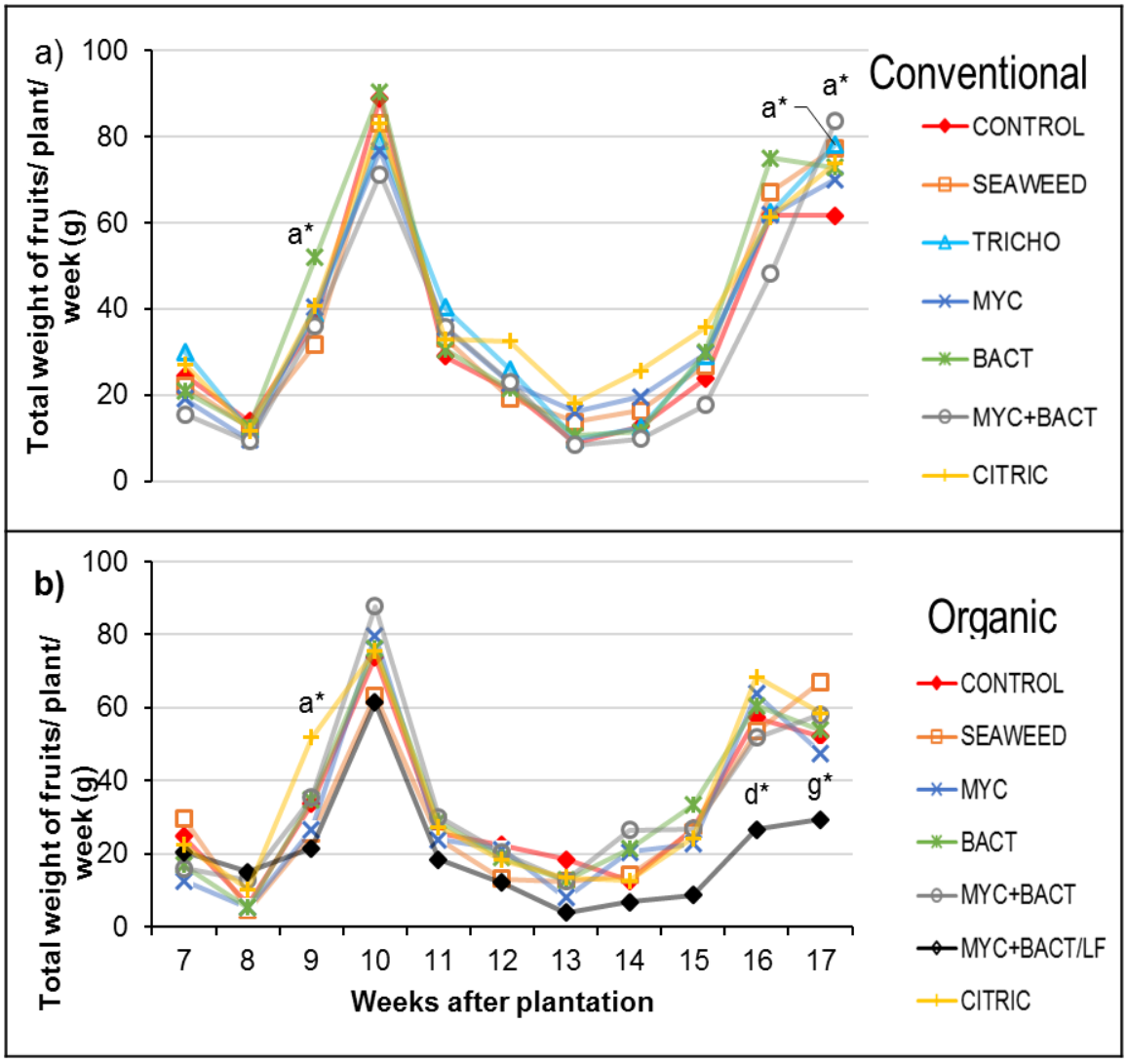

Figure 3.13 Influences of time of harvest on total weight of fruits from strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

Table 3.7 Yield parameters of strawberry plants cultivated in greenhouse under conventional and organic growing systems and biostimulant treatments during 11 harvest week (n=55).

Treatments		Total number of fruits/ plant/weeks	Total weight of fruits/ plant/week (g)	Number of marketable fruits/ plant/weeks	Weight of marketable fruits/ plant/week (g)	Number of unmarketable fruits/ plant/weeks	Weight of unmarketable fruits/ plant/week (g)
Conventional	**CONTROL**[z]	2.69 bc[x]	35.84 bc	2.43 bc	34.87 bcd	0.265 bcd	0.887 bcd
	SEAWEED	2.75 bc	37.43 abc	2.55 b	36.67 abc	0.193 cde	0.880 cde
	TRICHO	2.95 b	39.13 ab	2.67 b	38.02 ab	0.273 bc	0.883 abc
	MYC	2.72 bc	37.26 abc	2.52 bcd	36.52 abc	0.200 cde	0.934 cde
	BACT	2.76 bc	39.68 ab	2.56 b	38.88 ab	0.200 cde	0.934 cde
	MYC+BACT	2.37 d*	33.32 cd	2.20 d*	32.65 cde	0.169 de	0.845 de
	CITRIC	3.21 a*	41.36 a*	2.91 a*	40.24 a*	0.305 ab	0.998 ab
Organic	**CONTROL**	2.65 bc	33.41 cd	2.35 bc	32.16 cde	0.316 abc	0.908 abc
	SEAWEED	2.48 cd	31.09 d	2.22 cd	30.30 bcd	0.223 bcd	0.981 bcd
	MYC	2.40 cd	30.86 d	2.16 cd	30.17 cd	0.195 cd	0.847 cde
	BACT	2.64 bc	34.01 cd	2.39 bc	33.01 bcd	0.240 bcd	0.954 bcd
	MYC+BACT	3.00 ab	35.83 bc	2.62 ab	34.43 a*	0.387 a	1.058 a
	MYC+BACT/LF	1.43 e*	20.83 e*	1.32 e*	20.40 e	0.110 e*	0.886 e*
	CITRIC	3.06 b	36.43 bc	2.61 b	34.82 a*	0.430 a	0.962 a
Growing systems	Conventional	37.72 a	2.78 a	2.55 a	36.84 a	0.23	0.91 a
	Organic	31.78 b	2.52 b	2.24 b	30.76 b	0.27	0.94 b
P values							
Biostimulant (B)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time (T)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
B × T		0.093	0.004	0.197	0.002	0.003	0.017
Conventional vs Organic		0.001	<0.001	<0.001	<0.001	0.104	0.119

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).
[x]means of the same column with different letters are significantly different at P<0.05.
*Treatments are different from their respective control.

3.1.6.2 Marketable fruits

The results of the number and weight of marketable fruits are presented in Table 3.7. The marketable fruits (number of marketable fruits and weight of marketable fruits) were significantly influenced by the application of biostimulants (P<0.001), time of harvest (P<0.001), and growing systems (P<0.001). Although no significant interaction was observed for the number of marketable fruits, a significant interaction between biostimulant treatment and time was observed for the weight of marketable fruits (P=0.002) (Figure 3.14).

For conventional growing system, the number of marketable fruits were significantly increased by foliar application of citric acid (+20%) compared with the control, while the mixture of mycorrhiza and bacteria (MYC+BACT) reduced the number of marketable fruits compared with the control (-9%). No significant difference was observed between the other biostimulants and the control (Table 3.7). Under the organic management, biostimulant did not increase the number of marketable fruits compared to the control. However, the number of marketable fruits per week was reduced (-44%) in the low fertilization (MYC+BACT/LF) treatment compared to the organic control.

For the marketable fruit weight (marketable yield) of both growing systems, there was a significant difference between treatments at weeks 9 and 17 after plantation, and at week 16 for the organic system. At week 9, conventional plants treated with a combination of bacteria had +40% higher marketable yield compared with the control, while at week 17 plants treated with the mixture of mycorrhiza and bacteria (MYC+BACT) and *Trichoderma* (TRICHO) produced higher weight of marketable fruits (+36% and +27%, respectively) compared with the control (Figure 3.14a). Under organic management, plants treated with citric acid at week 9 produced higher marketable yield (+50%) compared with the control (Fig. 13.4b). The mixture of mycorrhiza and bacteria with low fertilization (MYC+BACT/LF) had the lowest value for the weight of marketable fruits compared with the control (-43%) (Figure 3.14b).

On the other hand, when the cumulative marketable fruit weight is considered (seasonal fruit weight without time effect), the foliar application of citric acid for conventional and organic plants significantly augmented the weight of marketable fruits compared to the control (+15% and 8%, respectively). In an organic system, treatments with the

combination of mycorrhiza and bacteria increased the marketable fruit weight by 7% compared with control.

Regardless of the biostimulant treatments, conventionally-grown plants produced a higher number (+14%) and weight of marketable fruits (+20%) than organically-grown plants (Table 3.7).

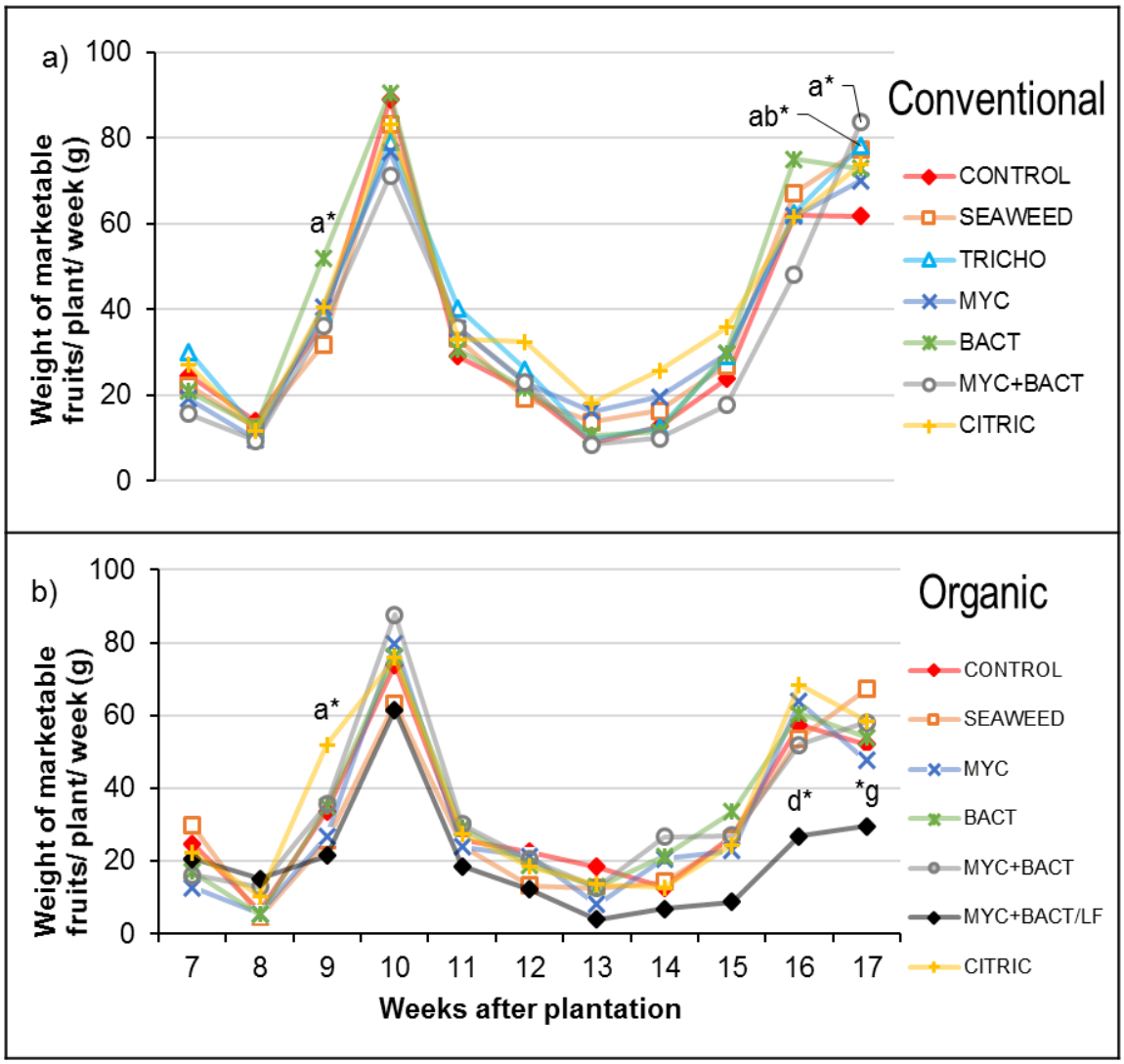

Figure 3.14 Influences of time on weight of marketable fruits from strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

3.1.6.3 Unmarketable fruits

Table 3.7 shows that unmarketable fruits were influenced by biostimulants (P<0.001) and time (P<0.001). However, an interaction between biostimulants and time was observed for the number (P=0.003) and weight (P=0.017) of unmarketable fruits. Significant effects between biostimulant treatments were observed at weeks 10 and 17. Like the total yield and marketable yield, in the conventional system, treatment with citric acid and *Trichoderma* produced a higher number (+108% and +138%, respectively) of and weight (+94% and +132%, respectively) of unmarketable fruits at week 17 after plantation compared with the control. At week 10 after plantation the combination of bacteria reduced (-77%) the number of unmarketable fruits, while the combination of bacteria and citric acid treatments decreased (-59% and -59%, respectively) the weight of unmarketable fruits of conventionally-grown plants (Fig. 3.15a, 3.16a). For organically-grown plants, citric acid increased the unmarketable yield at week 10 compared with the control, while at week 17 all biostimulant treatments, except citric acid, reduced the unmarketable yield (Fig. 3.15b, 3.16b).

When the cumulative fruit number and cumulative weight were considered without examining the time effect, no effect of biostimulants was observed compared with the control. Only the treatment with the low fertilization reduced the number and weight of unmarketable fruits compared with the other treatments (Table 3.7).

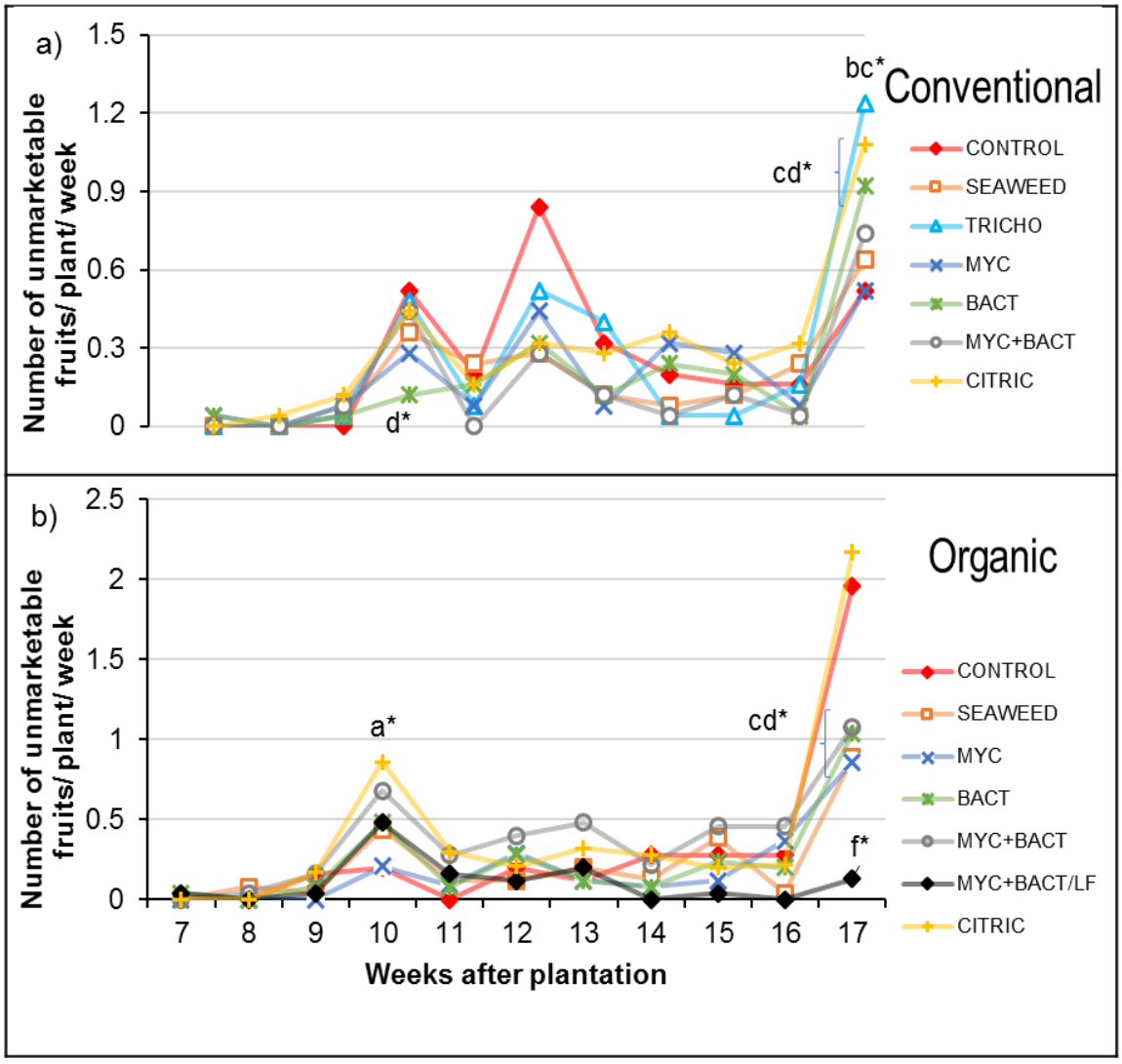

Figure 3.15 Influences of time on the number of unmarketable fruits from strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

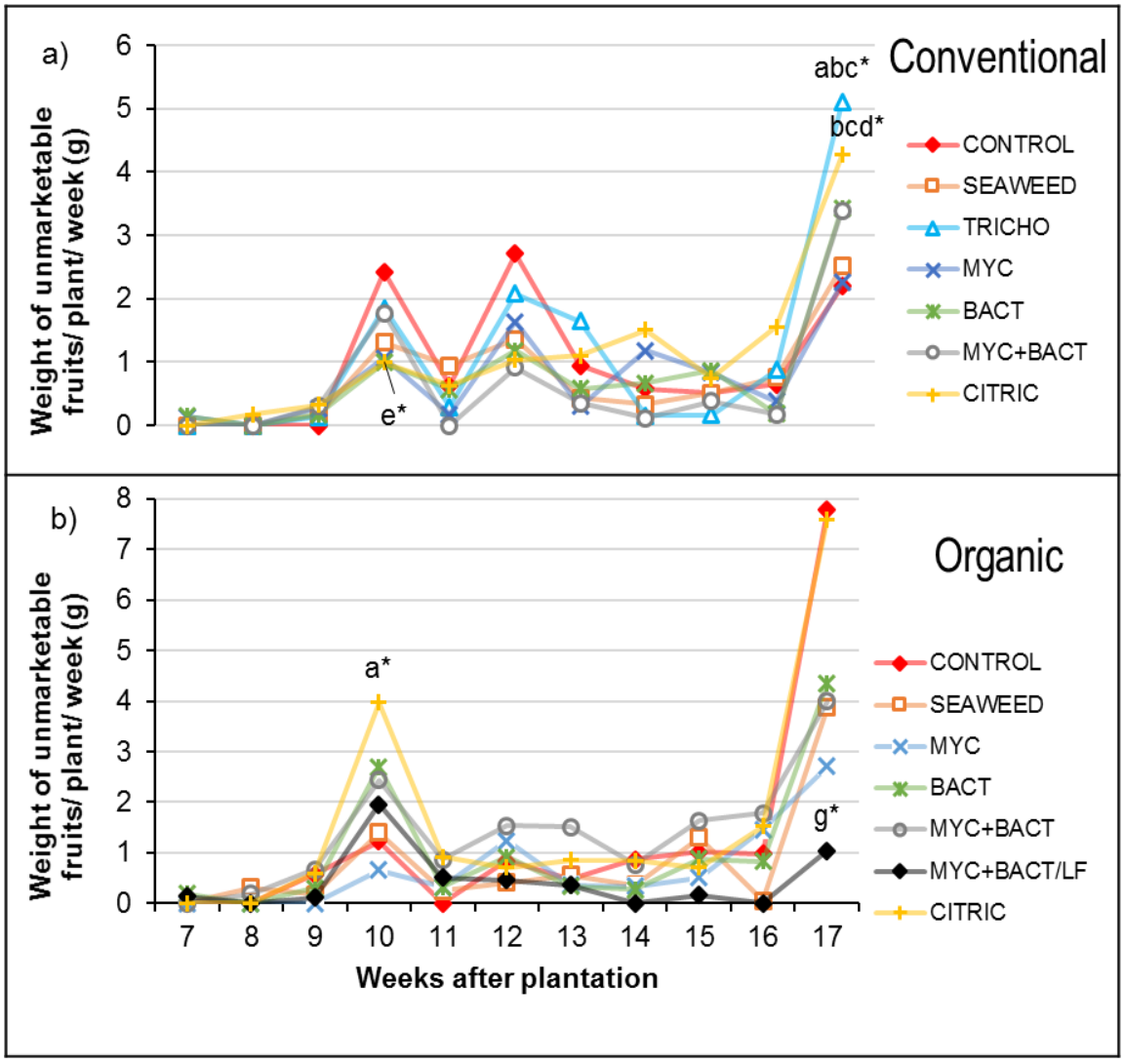

Figure 3.16 Influence of time on weight of unmarketable of fruits from strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5). *Treatments are different from their respective control.

3.1.7 Fruit quality

The fruit quality parameters evaluated during the greenhouse experiment were the total soluble sugars (Brix) and the total polyphenol and anthocyanin content. Significant treatment effects were observed for all parameters, although a significant interaction with time was observed for the total soluble sugar content (Tables 3.8 and 3.9).

3.1.7.1 Total soluble sugar level (°Brix)

Results in Table 3.8 show that the soluble sugar content (°Brix) was significantly influenced by the application of biostimulants (P<0.001) and time (P<0.001). However, a significant interaction between biostimulant treatments and time of measurement was observed (P=0.040). However, no significant difference was observed between the organic and conventional growing systems.

In the conventional system, the positive effect of biostimulants compared with the control was observed for treated plants with seaweed extract on May 1st (+4%) and *Trichoderma* (TRICHO) on April 15th (+23%) and May 1st (+19%). On the other hand, the lower soluble sugar content was observed for the treatments with a combination of bacteria (BACT) on May 1st (-12%) and the mixture of mycorrhiza and bacteria on May 15th (-15%). Other treatments did not show any significant difference with the control (Table 3.8). Treatment with a mixture of mycorrhiza and bacteria (MYC+BACT) for organically-grown plants increased the level of soluble sugar content on April 15th (+24%) and May 1st (+22%) compared with the control.

Table 3.8 Brix value of berries from strawberry plants cultivated in greenhouse under conventional and organic growing systems and biostimulant treatments (n=20).

Treatments		**°Brix**			
		April		**May**	
		1	**15**	**1**	**15**
Conventional	**CONTROL**[z]	7.28abcd[x]	9.4c	10.22cd	8.94ab
	SEAWEED	8.88a	9.8abc	10.58bc	8.48abc
	TRICHO	7.76abcd	11.54a*	12.16a*	8.425abc
	MYC	8.2abc	9.3c	10.38cd	8.18abc
	BACT	7.44abcd	7.44d*	8.96d	8.26abc
	MYC+BACT	7.16bcd	9.62bc	10.46bcd	7.6c*
	CITRIC	6.72cd	10.46abc	11.26abc	8.44abc
Organic	**CONTROL**	7.96abcd	9.04cd	9.8cd	8.64abc
	SEAWEED	8.72ab	9.94abc	10.68abc	8.28abc
	MYC	6.4d	9.32c	10.48bcd	8.52abc
	BACT	6.88cd	9.18cd	10.04cd	9.04ab
	MYC+BACT	7.9abcd	11.22ab*	11.92ab*	8.8ab
	MYC+BACT/LF	8.28abc	9.18cd	10.2cd	8bc
	CITRIC	7.92abcd	9.82abc	10.58bc	9.2a
P values					
Biostimulant (B)		<0.001			
Time (T)		<0.001			
B × T		0.040			
Conventional vs Organic		0.526			

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).

[x]means of the same column with different letters are significantly different at P<0.05.

*Treatments are different from their respective control.

3.1.7.2 Total phenolics assay and anthocyanins

Data presented the Table 3.9 show that there is a significant difference between treatments (*P= 0.0008*) and time of measurement (*P*= 0.0116) for the fruit total polyphenol content. No significant difference was observed between both growing systems and no interaction was observed between biostimulants and time.

Plants treated with *Trichoderma* (TRICHO) in the conventional system and with the mixture of mycorrhiza and bacteria (MYC+BACT) in the organic system produced fruits with a higher concentration of the total polyphenols compared with the control (+31% and +40%, respectively). However, the other treatments did not show any significant difference compared to the control. Regardless of the biostimulants, fruits harvested in May had 81% more phenol than fruits harvested in April.

The concentration of anthocyanins in strawberry fruits was also significantly influenced by the biostimulants (*P*=0.0052). There was no significant difference between the time of measurement and no interaction between treatments and time of measurement was observed. We also did not observe any significant difference between conventional and organic growing systems (Table 3.9).

In the conventional system, higher levels of anthocyanins were observed in berries treated with *Trichoderma* (+35%) and citric acid (+24%) compared with the control. For organically-grown plants, treatment with a mixture of mycorrhiza and bacteria (MYC+BACT) produced fruits with a higher concentration of anthocyanins (+25%) compared with the control (Table 3.9). Other treatments did not show any significant difference with the control.

Table 3.9 Total polyphenol and anthocyanin concentrations of strawberry fruits cultivated in greenhouse under different combination of growing systems and biostimulants (n=10).

Treatments		Total polyphenols (mg GAE /100gDW)	Anthocyanins (mg/100gDW)
Conventional	**CONTROL**[z]	6812b[x]	226 cd
	SEAWEED	7172b	240bcd
	TRICHO	8916a*	305a*
	MYC	6984b	274abc
	BACT	6082b	225cd
	MYC+BACT	6723b	271abc
	CITRIC	7271b	281ab*
Organic	**CONTROL**	6184b	240bcd
	SEAWEED	7018b	256abcd
	MYC	7101b	230bcd
	BACT	7202b	227cd
	MYC+BACT	8633a*	301a*
	MYC+BACT/LF	6841b	221d
	CITRIC	6772b	275abc
Growing systems	Conventional	7137	226
	Organic	7107	240
Time	April	5069b	305
	May	9176a	274
P values			
Biostimulant (B)		0.001	0.005
Time (T)		0.012	0.314
B × T		0.865	0.958
Conventional vs Organic		0.901	0.302

[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).

[x]means of the same column with different letters are significantly different at P<0.05.

*Treatments are different from their respective control.

The total polyphenolic contents, determined by the FC method, were expressed as mg of gallic acid equivalents (GAE) per 100 g of the dry weight of strawberry fruits (mg GAE 100 g^{-1} DW).

3.1.8 Principal component analysis (PCA)

A principal component analysis (PCA) was used to represent the relationship between some important variables and biostimulant treatments (Fig. 3.17). PC1 and PC2 explained 67.74% of the total variance, with accounting 38.11% and 29.63%, respectively (Figure 3.17). A clear clustering among both growing systems were observed. The conventional treatments (1 to 7) are all located in the left upper quadrat of the figure, while the organic treatments (8 to 14) are all located in the two right quadrats, except for the lower fertilization treatment (13), which is at the left lower quadrat.

Conventional treated plants were strongly related to a high P and Ca leaf content, and this was particularly true for the ones treated with citric acid, seaweed, and mycorrhiza. Organically-grown plants treated with the mixture of mycorrhiza and bacteria was strongly related to the leaf N content and SPAD value, while plants treated with citric acid was associated with leaf $N\text{-}NO_3$. On the other hand, organically-grown plants treated with mycorrhiza was strongly related to soil CO_2 efflux, FDA and leaf $N\text{-}NH_4$. These relationships were also observed, but with a lesser extent, for organically-grown plants treated with bacteria, seaweed extract and the control. For the lower fertilized plants, a negative relationship was observed for all variables.

The main variable of PC1 was $N\text{-}NO_3$, followed by N, SPAD, Mg, $N\text{-}NH_4$, FDA and CO_2 efflux. For PC2, the main variable was the marketable fruits followed by anthocyanins, P, total polyphenols, and Brix. The Ca leaf content was in opposite relationship with the leaf $N\text{-}NH_4$ content and the soil biological activity expressed by FDA and CO_2 efflux.

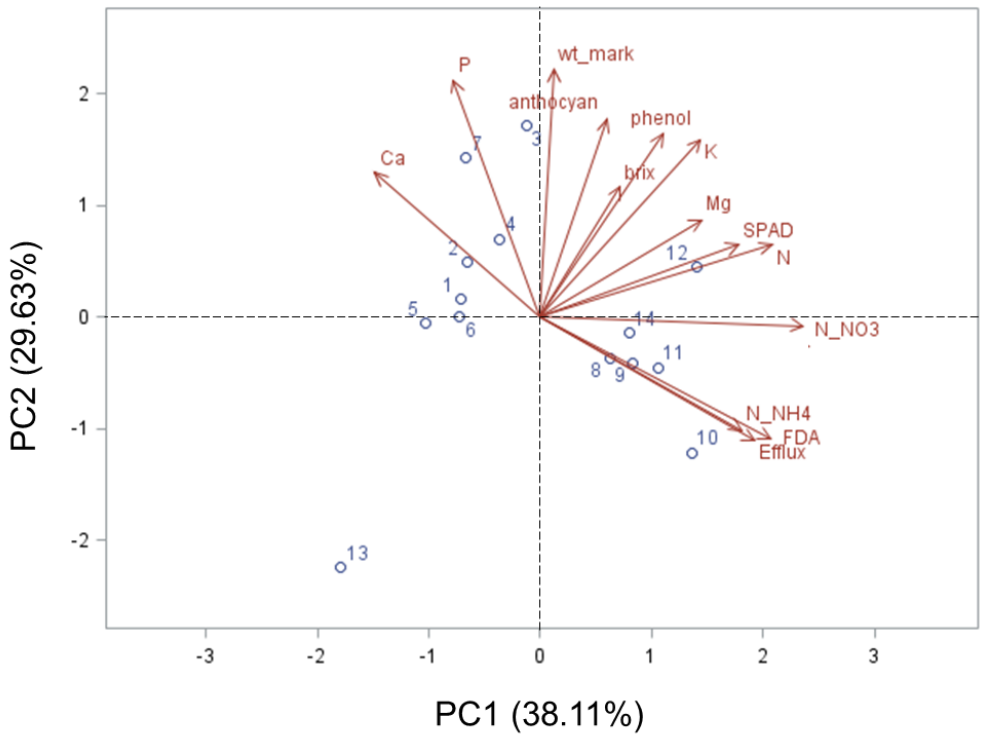

Figure 3.17 Relationships between leaf mineral content and physiological, yield, quality and soil activity parameters during the greenhouse experiment (PCA). 1: conventional control, 2: conventional seaweed extract, 3: conventional Trichoderma, 4: conventional mycorrhiza, 5: conventional combination of bacteria, 6: conventional mixture of mycorrhiza and bacteria, 7: conventional citric acid, 8: organic control, 9: organic seaweed, 10: organic mycorrhiza, 11: organic combination of bacteria, 12: organic mixture of mycorrhiza and bacteria, 13: organic mixture of mycorrhiza and bacteria with lower fertilization, 14: organic citric acid.

3.1.9 Relationship between soil activity and leaf mineral content, and the physiological, growth, yield and quality parameters

We have observed positive correlations between FDA and N-NO_3 (r= 0.744; *P*= 0.002) N-NH_4 (r= 0.0.708; *P*= 0.003), Na (r= 0.627; *P*= 0.012) and Zn (r= 0.874; *P*< 0.0001) level in the leaves and CO_2 efflux (r= 0.790; p= 0.001), while a negative correlation was observed with Ca (r= -0.751; *P*= 0.001), Fe (r=-0.851; *P*< 0.0001) and B (r=-0.819; *P*= 0.000). Similarly, there was a positive correlation between CO_2 efflux and N-NO_3 (r= 0.699; *P*= 0.004), N-NH_4 (r= 0.846; *P*= 0.004), Na (r=0.764; *P*= 0.001) and Zn (r=0.694; *P*= 0.004) level in the leaves, while it was negatively correlated with P (r= -0.658; *P*= 0.008), Ca (r= -0.621; *P*= 0.014), Fe (r= -0.703; *P*= 0.004) and B (r= -0.736; *P*= 0.002) (Table 3.10). The principal component analysis also showed that N-NH_4 level in the leaves, FDA, and CO_2

efflux are closely related (Figure 3.17). The abundance of soil bacteria and fungi was positively correlated with soil FDA (r= 0.876; *P*= 0.002) and CO_2 efflux (r= 0.704; *P*= 0.034) as well as leaf N (r= 0.744; *P*= 0.022), N-NH_4 (r= 0.668; *P*= 0.05) and N-NO_3 (r= 0.762; *P*= 0.017) content, while they were negatively correlated with leaf P (r=-0.899; p= 0.001), Ca (r= -0.829; *P*= 0.006), Fe (r= -0.951; *P*< 0.0001) and B (r= -0.951; *P*< 0.0001) content (Table 3.10).

Positive correlations between performance index with concentration of total nitrogen (r= 0.724; *P*= 0.002), N-NO_3 (r= 0.698; *P*=0.004) and potassium (r= 0.804; *P*= 0.000) in the leaves. Besides, we have observed a positive relationship between the chlorophyll content (SPAD) and total nitrogen (r= 0.725; *P*= 0.002), N-NO_3 (r= 0.72; *P*=0.003) and potassium (r= 0.732; p= 0.002) contents in the leaves. Principal component analysis also showed that the concentration of N in the leaves and the chlorophyll content (SPAD) are closely related (Figure 3.17). However, chlorophyll content was negatively correlated with themaximum rate of photosynthesis (Amax) (r= -0.538; *P*= 0.038) and quantum efficiency (r= -0.578; *P*= 0.024). The maximum rate of photosynthesis was positively correlated to concentration of Fe (r= 0.653; *P*= 0.008) and B (r= 0.675; *P*= 0.006), while it was negatively correlated with N-NH_4 (r= -.0754; *P*= 0.001), N-NO_3 (r= -0.647; *P*= 0.009), Na (r= -0.588; *P*= 0.021), and Zn (r= -0.716; *P*= 0.003) content.

The leaf concentration of potassium (r= 0.802; *P*= 0.000) and N (r= 0.516; *P*= 0.049) were positively correlated with the number of leaves (Table 3.10). The flowering stalks was positively correlated with Mg (r= 0.66; *P*= 0.007), while the number of crowns was correlated with P (r= 0.599; *P*= 0.018) and K (r= 0.849; *P*< 0.0001), and its crown diameter correlated with N (r= 0.524; *P*= 0.045) and K (r= 0.754; *P*= 0.001).

Shoot fresh biomass (r= 0.777; *P*= 0.001) and leaf area (r= 0.772; *P*= 0.001) was positively correlated with P leaf content, while it dry biomass was correlated with P (r= 0.79; *P*= 0.001) and K (r= 0.645; p= 0.01) leaf content. Similarly, root fresh biomass was correlated with P (r= 0.808; *P*=0.000) and K (r= 0.293; *P*= 0.289) leaf content, while root dry biomass was correlated with P leaf content (r= 0.809; *P*= 0.000).

Regarding the yield parameters, the total number of fruits was correlated with P (r= 0.621; *P*= 0.013), K (r= 0.761; *P*= 0.001) and Mg (r= 0.586; *P*= 0.022) leaf content, while the number of marketable fruits was correlated with leaf Mg content (r= 0.514; *P*= 0.037). Total and marketable yields were correlated with P (r= 0.833; *P*= 0.000; r= 0.854; *P*< 0.0001,

respectively) and Ca (r= 0.0.525; *P*= 0.045; r=0.543; *P*= 0.037, respectively), while the number (r= 0.605; *P*= 0.017) and weight (r= 0.603; *P*= 0.017) of unmarketable fruits was correlated with Mg. However, no correlation was observed between leaf macronutrients and the quality parameters, except for the fruit anthocyanins that was correlated with leaf Mg content (r= 0.517; *P*= 0.049) and soil CO_2 efflux (r= 0.747; *P*= 0.001).

Positive correlations were observed between Fv/Fm, PI and SPAD, which they were positively correlated to the number of leaves and crowns and the marketable fruit weight (Table 3.11). In addition, PI and SPAD were positively correlated with the crown diameter and total fruit number, while PI was positively correlated with leaf area, shoot fresh and dry weights, and the total fruit weights (Table 3.11).

The number of leaves and flowering stalks as well as the crown number and diameter were positively correlated with the total, marketable and unmarketable fruit number, and fruit weight, but they were not correlated to any quality parameters.

Shoot fresh and dry weights as well as the leaf area were positively correlated with total and marketable fruit number and weight, while root fresh and dry weights were positively correlated with total fruit number and weight. No correlation was observed between yield parameters, leaf area, and leaf mineral content (Table 3.11).

Table 3.10 Relationships between the mineral content of leaves and soil activity and physiological, growth, yield and quality parameters of greenhouse strawberry plants. The numbers in red are significant at P <0.05.

	N	P	K	Ca	Mg	$N\text{-}NH_4$	$N\text{-}NO_3$	Na	Fe	Zn	B
Soil activity											
FDA	0.610	-0.648	0.249	-0.751	0.347	0.708	0.744	0.627	-0.851	0.874	-0.819
P value	0.016	0.009	0.370	0.001	0.205	0.003	0.002	0.012	<.0001	<.0001	0.000
CO_2 efflux	0.399	-0.658	0.038	-0.621	0.343	0.846	0.699	0.764	-0.703	0.694	-0.736
P value	0.140	0.008	0.894	0.014	0.211	<.0001	0.004	0.001	0.004	0.004	0.002
Bacteria	0.744	-0.899	0.388	-0.829	0.516	0.668	0.762	0.635	-0.951	0.635	-0.951
P value	0.022	0.001	0.302	0.006	0.155	0.050	0.017	0.066	<.0001	0.066	<.0001
Fungi	0.782	-0.967	0.260	-0.789	0.671	0.793	0.895	0.739	-0.983	0.739	-0.983
P value	0.013	<.0001	0.500	0.012	0.048	0.011	0.001	0.023	<.0001	0.023	<.0001
Leaf mineral content											
N	1.000	0.007	0.688	-0.452	0.556	0.310	0.809	0.541	-0.264	0.832	-0.287
P value		0.980	0.005	0.091	0.032	0.261	0.000	0.037	0.341	0.000	0.299
P	0.007	1.000	0.487	0.658	0.010	-0.616	-0.282	-0.430	0.902	-0.423	0.917
P value	0.980		0.066	0.008	0.973	0.014	0.308	0.109	<.0001	0.116	<.0001
K	0.688	0.487	1.000	-0.115	0.408	-0.029	0.477	0.089	0.210	0.404	0.129
P value	0.005	0.066		0.683	0.131	0.918	0.072	0.751	0.454	0.135	0.648
Ca	-0.452	0.658	-0.115	1.000	0.146	-0.489	-0.593	-0.469	0.740	-0.591	0.808
P value	0.091	0.008	0.683		0.603	0.064	0.020	0.078	0.002	0.020	0.000
Mg	0.556	0.010	0.408	0.146	1.000	0.283	0.453	0.431	-0.122	0.642	-0.162
P value	0.032	0.973	0.131	0.603		0.307	0.090	0.109	0.666	0.010	0.564
$N\text{-}NH_4$	0.310	-0.616	-0.029	-0.489	0.283	1.000	0.692	0.815	-0.647	0.633	-0.676
P value	0.261	0.014	0.918	0.064	0.307		0.004	0.000	0.009	0.011	0.006
N_NO_3	0.809	-0.282	0.477	-0.593	0.453	0.692	1.000	0.749	-0.456	0.846	-0.516
P value	0.000	0.308	0.072	0.020	0.090	0.004		0.001	0.088	<.0001	0.049
Na	0.541	-0.430	0.089	-0.469	0.431	0.815	0.749	1.000	-0.583	0.751	-0.553
P value	0.037	0.109	0.751	0.078	0.109	0.000	0.001		0.023	0.001	0.033
Fe	-0.264	0.902	0.210	0.740	-0.122	-0.647	-0.456	-0.583	1.000	-0.665	0.928
P value	0.341	<.0001	0.454	0.002	0.666	0.009	0.088	0.023		0.007	<.0001
Zn	0.832	-0.423	0.404	-0.591	0.642	0.633	0.846	0.751	-0.665	1.000	-0.653
P value	0.000	0.116	0.135	0.020	0.010	0.011	<.0001	0.001	0.007		0.008
B	-0.287	0.917	0.129	0.808	-0.162	-0.676	-0.516	-0.553	0.928	-0.653	1.000
P value	0.299	<.0001	0.648	0.000	0.564	0.006	0.049	0.033	<.0001	0.008	

	N	P	K	Ca	Mg	$N\text{-}NH_4$	$N\text{-}NO_3$	Na	Fe	Zn	B
Physiological parameters											
Fv/Fm	0.390	-0.116	0.627	-0.440	0.362	0.349	0.487	0.258	-0.213	0.468	-0.451
P value	0.150	0.680	0.012	0.101	0.185	0.203	0.066	0.354	0.446	0.079	0.092
PI	0.724	0.056	0.804	-0.497	0.374	0.333	0.698	0.324	-0.096	0.604	-0.303
P value	0.002	0.843	0.000	0.060	0.170	0.225	0.004	0.239	0.733	0.017	0.273
SPAD	0.725	0.179	0.732	-0.405	0.198	0.424	0.720	0.498	-0.067	0.534	-0.134
P value	0.002	0.522	0.002	0.134	0.480	0.116	0.003	0.059	0.813	0.040	0.635
A_{max}	-0.413	0.476	-0.295	0.509	-0.366	-0.754	-0.647	-0.588	0.653	-0.716	0.672
P value	0.126	0.073	0.286	0.053	0.180	0.001	0.009	0.021	0.008	0.003	0.006
Rd	0.449	-0.156	0.243	-0.457	0.019	0.090	0.497	0.227	-0.224	0.376	-0.233
P value	0.093	0.579	0.383	0.087	0.946	0.750	0.060	0.415	0.421	0.168	0.404
Φ	-0.464	-0.066	-0.303	0.403	0.061	-0.181	-0.603	-0.329	-0.009	-0.306	0.043
P value	0.081	0.816	0.273	0.137	0.829	0.518	0.017	0.231	0.975	0.267	0.879
Growth parameters											
Number of leaves	0.516	0.509	0.820	0.129	0.464	0.185	0.444	0.17	0.304	0.323	0.214
P value	0.049	0.053	0.000	0.647	0.082	0.508	0.098	0.544	0.271	0.241	0.444
Number of flowering stalks	0.436	0.383	0.617	0.315	0.660	0.107	0.323	0.054	0.257	0.324	0.156
P value	0.105	0.158	0.014	0.252	0.007	0.704	0.240	0.847	0.356	0.238	0.580
Number of crowns	0.464	0.599	0.849	0.198	0.458	0.010	0.338	0.070	0.368	0.266	0.301
P value	0.081	0.018	<.0001	0.480	0.086	0.973	0.217	0.803	0.177	0.339	0.276
Diameter of crowns	0.524	0.433	0.754	0.092	0.416	0.186	0.473	0.132	0.290	0.275	0.168
P value	0.045	0.107	0.001	0.745	0.123	0.507	0.075	0.639	0.295	0.321	0.549
Shoot fresh biomass	0.312	0.777	0.670	0.202	-0.067	-0.247	0.067	-0.084	0.546	-0.066	0.596
P value	0.257	0.001	0.006	0.471	0.813	0.375	0.813	0.765	0.035	0.815	0.019
Shoot dry biomass	0.300	0.79	0.645	0.248	-0.042	-0.253	0.084	-0.091	0.553	-0.061	0.623
P value	0.277	0.001	0.010	0.373	0.882	0.362	0.767	0.746	0.033	0.828	0.013
Root fresh biomass	-0.196	0.808	0.293	0.542	-0.272	-0.485	-0.305	-0.507	0.755	-0.488	0.821
P value	0.485	0.000	0.289	0.037	0.326	0.067	0.269	0.054	0.001	0.065	0.000
Root dry biomass	-0.175	0.809	0.305	0.501	-0.320	-0.463	-0.280	-0.496	0.757	-0.491	0.813
P value	0.533	0.000	0.269	0.057	0.245	0.082	0.313	0.060	0.001	0.063	0.000
Leaf area	0.421	0.772	0.762	0.126	0.014	-0.26	0.189	-0.035	0.499	0.040	0.537
P value	0.118	0.001	0.001	0.656	0.960	0.350	0.500	0.901	0.058	0.886	0.039

	N	P	K	Ca	Mg	$N\text{-}NH_4$	$N\text{-}NO_3$	Na	Fe	Zn	B
Yield parameters											
Total number of fruits	0.490	0.621	0.761	0.323	0.586	0.040	0.319	0.103	0.439	0.278	0.363
P value	0.064	0.013	0.001	0.240	0.022	0.887	0.247	0.715	0.102	0.317	0.183
Number of marketable fruits	0.456	0.719	0.735	0.409	0.541	-0.063	0.248	0.049	0.542	0.186	0.484
P value	0.088	0.003	0.002	0.130	0.037	0.825	0.373	0.862	0.037	0.507	0.067
Weight of marketable fruits	0.299	0.854	0.610	0.543	0.360	-0.236	0.055	-0.092	0.710	-0.035	0.681
P value	0.278	<.0001	0.016	0.037	0.188	0.397	0.845	0.745	0.003	0.900	0.005
Number of unmarketable fruits	0.453	0.052	0.598	-0.050	0.605	0.355	0.439	0.203	-0.082	0.520	-0.196
P value	0.090	0.855	0.019	0.860	0.017	0.194	0.101	0.469	0.772	0.047	0.483
Weight of unmarketable fruits	0.475	0.075	0.586	-0.029	0.603	0.325	0.428	0.195	-0.041	0.496	-0.177
P value	0.074	0.789	0.022	0.917	0.017	0.237	0.111	0.487	0.886	0.060	0.528
Total weight	0.320	0.833	0.629	0.525	0.387	-0.208	0.081	-0.077	0.686	-0.003	0.650
P value	0.244	0.000	0.012	0.045	0.154	0.457	0.775	0.786	0.005	0.992	0.009
Quality parameters											
Brix	0.216	0.040	0.389	0.020	0.445	-0.016	0.156	-0.192	0.150	0.166	-0.074
P value	0.439	0.888	0.152	0.943	0.097	0.956	0.579	0.493	0.593	0.554	0.792
Total polyphenols	0.354	0.200	0.376	0.094	0.533	0.087	0.364	0.070	0.314	0.230	0.104
P value	0.196	0.476	0.167	0.739	0.041	0.757	0.183	0.804	0.254	0.409	0.711
Anthocyanins	0.288	0.345	0.491	0.233	0.517	-0.076	0.061	-0.073	0.340	0.161	0.239
P value	0.298	0.208	0.063	0.404	0.049	0.789	0.830	0.795	0.215	0.567	0.391

Table 3.10 (continuity) Relationships between soil activity, physiological, growth, yield, and quality parameters (Winter 2018).

	FDA	CO_2 efflux	Fv/Fm	PI	SPAD	Nb leaves	Nb flowering stalks	Crown nb	Crown diameter
Soil activity									
FDA	1.000	0.790	0.462	0.483	0.452	0.147	0.071	0.040	0.159
P value		0.001	0.083	0.068	0.091	0.602	0.802	0.887	0.571
CO_2 efflux	0.790	1.000	0.379	0.354	0.303	0.018	-0.124	-0.152	0.044
P value	0.001		0.164	0.196	0.272	0.950	0.660	0.589	0.876
Bacteria	0.876	0.704	0.732	0.793	0.809	0.252	0.096	-0.201	0.326
P value	0.002	0.034	0.025	0.011	0.008	0.512	0.805	0.604	0.235
Fungi	0.918	0.776	0.788	0.849	0.697	0.280	0.249	-0.138	0.621
P value	0.001	0.014	0.012	0.004	0.037	0.466	0.518	0.723	0.075
Physiological parameters									
Fv/Fm	0.462	0.204	1.000	0.834	0.522	0.550	0.452	0.542	0.469
P value	0.083	0.465		0.000	0.046	0.034	0.091	0.037	0.078
PI	0.483	0.184	0.834	1.000	0.781	0.716	0.540	0.672	0.664
P value	0.068	0.511	0.000		0.001	0.003	0.038	0.006	0.007
SPAD	0.452	0.312	0.522	0.781	1.000	0.769	0.427	0.666	0.781
P value	0.091	0.257	0.046	0.001		0.001	0.112	0.007	0.001
A_{max}	-0.810	-0.572	-0.487	-0.510	-0.538	-0.399	-0.355	-0.335	-0.364
P value	0.000	0.026	0.066	0.052	0.038	0.141	0.194	0.222	0.182
Rd	0.258	0.081	-0.221	0.364	0.295	0.043	0.030	0.138	0.076
P value	0.354	0.774	0.429	0.183	0.286	0.878	0.914	0.625	0.789
Φ	-0.165	-0.106	0.195	-0.451	-0.578	-0.264	-0.048	-0.275	-0.325
P value	0.557	0.708	0.487	0.091	0.024	0.342	0.865	0.322	0.237
Growth parameters									
Number of leaves	0.147	0.018	0.550	0.716	0.769	1.000	0.826	0.953	0.931
P value	0.602	0.950	0.034	0.003	0.001		0.000	<.0001	<.0001
Nb flowering stalks	0.071	-0.124	0.452	0.540	0.427	0.826	1.000	0.834	0.748
P value	0.802	0.660	0.091	0.038	0.112	0.000		0.000	0.001
Number of crowns	0.040	-0.152	0.542	0.672	0.666	0.953	0.834	1.000	0.827
P value	0.887	0.589	0.037	0.006	0.007	<.0001	0.000		0.000
Diameter of crowns	0.159	0.044	0.469	0.664	0.781	0.931	0.748	0.827	1.000
P value	0.571	0.876	0.078	0.007	0.001	<.0001	0.001	0.000	

	FDA	CO_2 efflux	Fv/Fm	PI	SPAD	Nb leaves	Nb flowering stalks	Crown nb	Crown diameter
Shoot fresh biomass	-0.244	-0.356	0.079	0.389	0.574	0.666	0.379	0.717	0.525
P value	0.381	0.193	0.780	0.152	0.025	0.007	0.164	0.003	0.045
Shoot dry biomass	-0.239	-0.375	0.031	0.350	0.574	0.695	0.406	0.740	0.571
P value	0.391	0.169	0.914	0.201	0.025	0.004	0.134	0.002	0.026
Root fresh biomass	-0.58	-0.649	-0.216	-0.010	0.107	0.424	0.284	0.516	0.310
P value	0.023	0.009	0.439	0.973	0.704	0.115	0.304	0.049	0.261
Root dry biomass	-0.569	-0.644	-0.206	0.018	0.165	0.438	0.271	0.514	0.340
P value	0.027	0.010	0.462	0.950	0.556	0.103	0.329	0.050	0.216
Leaf area	-0.156	-0.292	0.183	0.471	0.619	0.691	0.398	0.752	0.552
P value	0.579	0.291	0.515	0.076	0.014	0.004	0.142	0.001	0.033
Yield parameters									
Total number of fruits	-0.007	-0.128	0.428	0.589	0.601	0.917	0.870	0.925	0.816
P value	0.980	0.650	0.111	0.021	0.018	<.0001	<.0001	<.0001	0.000
Nb marketable fruits	-0.119	-0.211	0.320	0.502	0.559	0.876	0.820	0.891	0.787
P value	0.673	0.450	0.244	0.056	0.031	<.0001	0.000	<.0001	0.001
Weight marketable fruits	-0.335	-0.385	0.119	0.323	0.455	0.779	0.689	0.797	0.696
P value	0.222	0.156	0.672	0.240	0.088	0.001	0.005	0.000	0.004
Nb unmarketable fruits	0.388	0.138	0.657	0.695	0.524	0.779	0.846	0.776	0.690
P value	0.153	0.625	0.008	0.004	0.045	0.001	<.0001	0.001	0.004
Weight unmarketable fruits	0.335	0.091	0.633	0.701	0.540	0.790	0.875	0.774	0.722
P value	0.222	0.747	0.011	0.004	0.038	0.001	<.0001	0.001	0.002
Total weight	-0.304	-0.360	0.156	0.358	0.476	0.805	0.724	0.821	0.720
P value	0.271	0.188	0.580	0.191	0.073	0.000	0.002	0.000	0.002
Quality parameters									
Brix	0.046	0.073	0.484	0.478	-0.044	0.239	0.471	0.289	0.211
P value	0.870	0.796	0.068	0.072	0.877	0.391	0.076	0.296	0.450
Total polyphenols	-0.045	0.180	0.320	0.492	0.122	0.344	0.452	0.345	0.329
P value	0.875	0.520	0.244	0.062	0.665	0.209	0.091	0.209	0.231
Anthocyanins	-0.026	-0.026	0.263	0.438	0.108	0.448	0.510	0.509	0.326
P value	0.928	0.928	0.344	0.102	0.703	0.094	0.052	0.053	0.235

Table 3.11 Relationships between physiological, growth, yield and quality parameters (Winter 2018).

	Shoot FW	Shoot DW	Root FW	Root DW	Leaf area	Total fruit nb	Mark. fruit nb
Physiological parameters							
Fv/Fm	0.079	0.031	-0.216	-0.206	0.183	0.428	0.320
P value	0.780	0.914	0.439	0.462	0.515	0.111	0.244
PI	0.389	0.350	-0.010	0.018	0.471	0.589	0.502
P value	0.152	0.201	0.973	0.950	0.076	0.021	0.056
SPAD	0.574	0.574	0.107	0.165	0.619	0.601	0.559
P value	0.025	0.025	0.704	0.556	0.014	0.018	0.031
A_{max}	0.133	0.117	0.375	0.358	0.095	-0.278	-0.147
P value	0.635	0.678	0.168	0.190	0.738	0.316	0.601
Rd	0.146	0.158	0.052	0.050	0.178	-0.026	-0.069
P value	0.603	0.575	0.854	0.861	0.526	0.926	0.808
Φ	-0.307	-0.351	-0.189	-0.227	-0.357	-0.191	-0.182
P value	0.265	0.199	0.499	0.417	0.192	0.496	0.517
Growth parameters							
Number of leaves	0.666	0.695	0.424	0.438	0.691	0.917	0.876
P value	0.007	0.004	0.115	0.103	0.004	<.0001	<.0001
Number of flowering stalks	0.379	0.406	0.284	0.271	0.398	0.870	0.820
P value	0.164	0.134	0.304	0.329	0.142	<.0001	0.000
Number of crowns	0.717	0.740	0.516	0.514	0.752	0.925	0.891
P value	0.003	0.002	0.049	0.050	0.001	<.0001	<.0001
Diameter of crowns	0.525	0.571	0.310	0.340	0.552	0.816	0.787
P value	0.045	0.026	0.261	0.216	0.033	0.000	0.001
Shoot fresh biomass	1.000	0.981	0.773	0.792	0.964	0.621	0.659
P value		<.0001	0.001	0.000	<.0001	0.014	0.008
Shoot dry biomass	0.981	1.000	0.816	0.831	0.959	0.635	0.675
P value	<.0001		0.000	0.000	<.0001	0.011	0.006
Root fresh biomass	0.773	0.816	1.000	0.994	0.704	0.421	0.474
P value	0.001	0.000		<.0001	0.003	0.118	0.074
Root dry biomass	0.792	0.831	0.994	1.000	0.720	0.426	0.481
P value	0.000	0.000	<.0001		0.003	0.114	0.069
Leaf area	0.964	0.959	0.704	0.720	1.000	0.638	0.678
P value	<.0001	<.0001	0.003	0.003		0.011	0.006

	Shoot FW	Shoot DW	Root FW	Root DW	Leaf area	Total fruit nb	Mark. fruit nb
Yield parameters							
Total number of fruits	0.621	0.635	0.421	0.426	0.638	1.000	0.987
P value	0.014	0.011	0.118	0.114	0.011		<.0001
Number of marketable fruits	0.659	0.675	0.474	0.481	0.678	0.987	1.000
P value	0.008	0.006	0.074	0.069	0.006	<.0001	
Weight of marketable fruits	0.720	0.744	0.610	0.622	0.720	0.906	0.958
P value	0.003	0.002	0.016	0.013	0.003	<.0001	<.0001
Number of unmarketable fruits	0.249	0.267	0.102	0.093	0.241	0.743	0.629
P value	0.371	0.336	0.716	0.743	0.386	0.002	0.012
Weight of unmarketable fruits	0.263	0.281	0.099	0.094	0.251	0.750	0.642
P value	0.344	0.311	0.725	0.740	0.367	0.001	0.010
Total weight	0.715	0.740	0.598	0.609	0.714	0.926	0.970
P value	0.003	0.002	0.019	0.016	0.003	<.0001	<.0001
Quality parameters							
Brix	1.000	-0.059	-0.049	-0.049	-0.292	0.360	0.303
P value		0.836	0.863	0.863	0.290	0.187	0.272
Total polyphenols	0.084	0.049	0.063	0.047	0.115	0.482	0.466
P value	0.767	0.861	0.824	0.868	0.684	0.069	0.080
Anthocyanins	0.377	0.314	0.277	0.240	0.305	0.574	0.543
P value	0.167	0.255	0.317	0.390	0.269	0.025	0.037

Table 3.11 (continuity) Relationships between physiological, growth, yield and quality parameters (Winter 2018).

	Mark. Fruit weight	Unmark. fruit nb	Umark fruit weight	Total fruit weight	Brix	Total polyphenols	Antocyanins
Physiological parameters							
Fv/Fm	0.119	0.657	0.633	0.156	0.484	0.320	0.263
P value	0.672	0.008	0.011	0.580	0.068	0.244	0.344
PI	0.323	0.695	0.701	0.358	0.478	0.492	0.438
P value	0.240	0.004	0.004	0.191	0.072	0.062	0.102
SPAD	0.455	0.524	0.540	0.476	-0.044	0.122	0.108
P value	0.088	0.045	0.038	0.073	0.877	0.665	0.703
A_{max}	0.081	-0.677	-0.618	0.035	-0.078	0.047	0.058
P value	0.775	0.006	0.014	0.900	0.782	0.869	0.837
Rd	-0.175	0.193	0.190	-0.147	0.176	0.256	0.181
P value	0.532	0.490	0.497	0.602	0.530	0.357	0.519
Φ	-0.143	-0.164	-0.168	-0.157	0.025	-0.296	-0.035
P value	0.611	0.559	0.549	0.577	0.929	0.283	0.903
Growth parameters							
Number of leaves	0.779	0.779	0.790	0.805	0.239	0.344	0.448
P value	0.001	0.001	0.001	0.000	0.391	0.209	0.094
Number of flowering stalks	0.689	0.846	0.875	0.724	0.471	0.452	0.510
P value	0.005	<.0001	<.0001	0.002	0.076	0.091	0.052
Number of crowns	0.797	0.776	0.774	0.821	0.289	0.345	0.509
P value	0.000	0.001	0.001	0.000	0.296	0.209	0.053
Diameter of crowns	0.696	0.690	0.722	0.720	0.211	0.329	0.326
P value	0.004	0.004	0.002	0.002	0.450	0.231	0.235
Shoot fresh biomass	0.720	0.249	0.263	0.715	-0.059	0.084	0.377
P value	0.003	0.371	0.344	0.003	0.836	0.767	0.167
Shoot dry biomass	0.744	0.267	0.281	0.740	-0.137	0.049	0.314
P value	0.002	0.336	0.311	0.002	0.628	0.861	0.255
Root fresh biomass	0.610	0.102	0.099	0.598	-0.049	0.063	0.277
P value	0.016	0.716	0.725	0.019	0.863	0.824	0.317
Root dry biomass	0.622	0.093	0.094	0.609	-0.071	0.047	0.240
P value	0.013	0.743	0.740	0.016	0.802	0.868	0.390
Leaf area	0.720	0.241	0.251	0.714	-0.049	0.115	0.305
P value	0.003	0.386	0.367	0.003	0.863	0.684	0.269

	Mark. Fruit weight	Unmark. fruit nb	Umark fruit weight	Total fruit weight	Brix	Total polyphenols	Antocyanins
Yield parameters							
Total number of fruits	0.906	0.743	0.750	0.926	0.360	0.482	0.574
P value	<.0001	0.002	0.001	<.0001	0.187	0.069	0.025
Number of marketable fruits	0.958	0.629	0.642	0.970	0.303	0.466	0.543
P value	<.0001	0.012	0.010	<.0001	0.272	0.080	0.037
Weight of marketable fruits	1.000	0.425	0.452	0.998	0.142	0.359	0.444
P value		0.114	0.091	<.0001	0.615	0.188	0.098
Number of unmarketable fruits	0.425	1.000	0.987	0.475	0.489	0.383	0.536
P value	0.114		<.0001	0.074	0.064	0.158	0.040
Weight of unmarketable fruits	0.452	0.987	1.000	0.502	0.471	0.384	0.525
P value	0.091	<.0001		0.057	0.077	0.158	0.045
Total weight	0.998	0.475	0.502	1.000	0.167	0.373	0.464
P value	<.0001	0.074	0.057		0.552	0.171	0.082
Quality parameters							
Brix	0.142	0.489	0.471	0.167	1.000	0.817	0.743
P value	0.615	0.064	0.077	0.552		0.000	0.002
Total polyphenols	0.359	0.383	0.384	0.373	0.817	1.000	0.747
P value	0.188	0.158	0.158	0.171	0.000		0.001
Anthocyanins	0.444	0.536	0.525	0.464	0.743	0.747	1.000
P value	0.098	0.040	0.045	0.082	0.002	0.001	

3.2 HIGH TUNNEL EXPERIMENT- SUMMER 2018

3.2.1 Microbial activity

The microbial activity of soils, expressed by the hydrolysis of the fluorescence diacetate (FDA), was influenced by the biostimulant treatments at *P*=0.082. The microbial activity was higher (+42% in average) in growing media of plants treated with a mixture of the mycorrhiza and bacteria (*P*=0.039) compared with the control and the other treatments (Figure 3.18). No statistical difference was observed between the other treatments and the control plants. The time of sampling had no significant effect on the microbial activity of the soil (*P*=0.296). Besides, no significant interaction was observed between treatments and time (*P*=0.403).

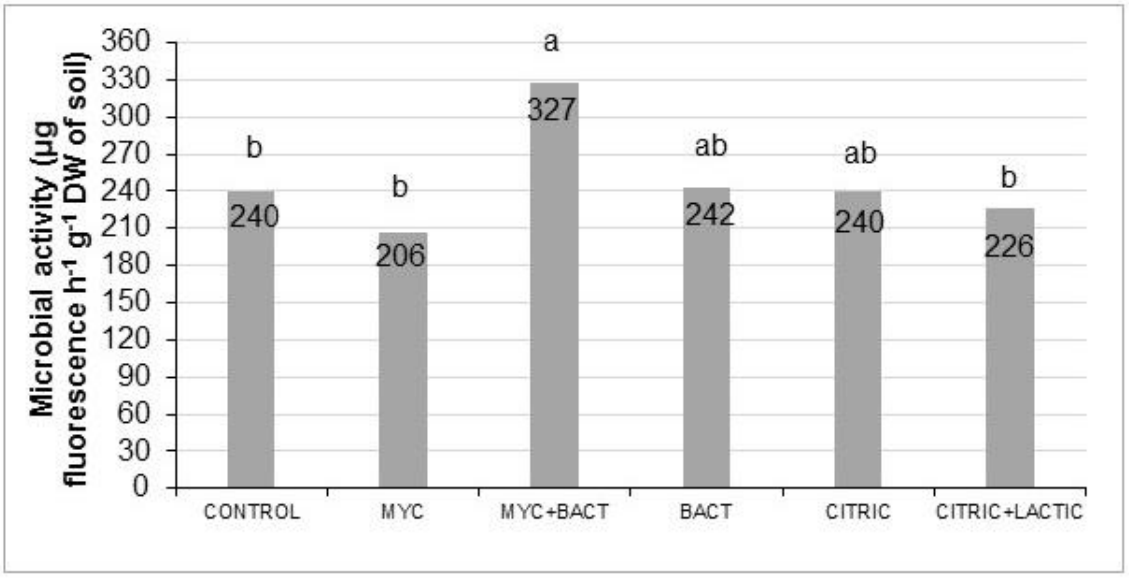

Figure 3.18 Effects of studied biostimulants on the microbial activity of the soil. Means followed by the same letter are not significantly different (P ≤ 0.05). Samples were collected in July 13th and October 24th (n=8).

3.2.2 Plant physiological parameters

3.2.2.1 Chlorophyll fluorescence

Chlorophyll fluorescence parameters were not significantly influenced by the biostimulant treatments (Table 3.12). However, both parameters, the maximum quantum efficiency of photosystem II (Fv/Fm) and performance index (P Index), the significant difference was observed for the time of measurement. Figures 3.19 and 3.20 indicated that the highest value of Fv/Fm and P Index was observed in September.

Table 3.12 The influence of the biostimulant treatments on the leaf chlorophyll fluorescence parameters (Fv/Fm and P Index) and leaf chlorophyll content of strawberry plants grown under high tunnel (n=36).

Treatments	**Fv/Fm**	**P Index**	**Chlorophyll content (SPAD unit)**
CONTROL[z]	0.821[x]	3.48	39.9
MYC	0.824	3.95	40.4
MYC+BACT	0.821	3.53	40.6
BACT	0.824	3.68	39.1
CITRIC	0.820	3.34	38.7
CITRIC+LACTIC	0.821	4.08	39.3
P values			
Biostimulant (B)	0.910	0.150	0.139
Time (T)	0.004	<0.001	0.014
B × T	0.968	0.956	0.632

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).
[x]means of the same column with different letters are significantly different at $P<0.05$.

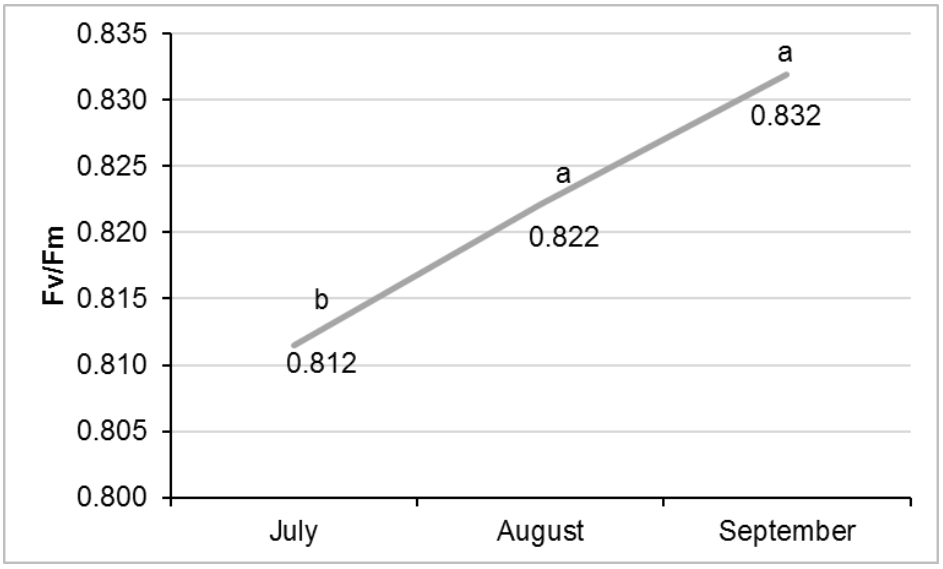

Figure 3.19 The maximum quantum efficiency of photosystem II (Fv/Fm) variation on strawberry leaves of plants during the experimental period of summer 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=72).

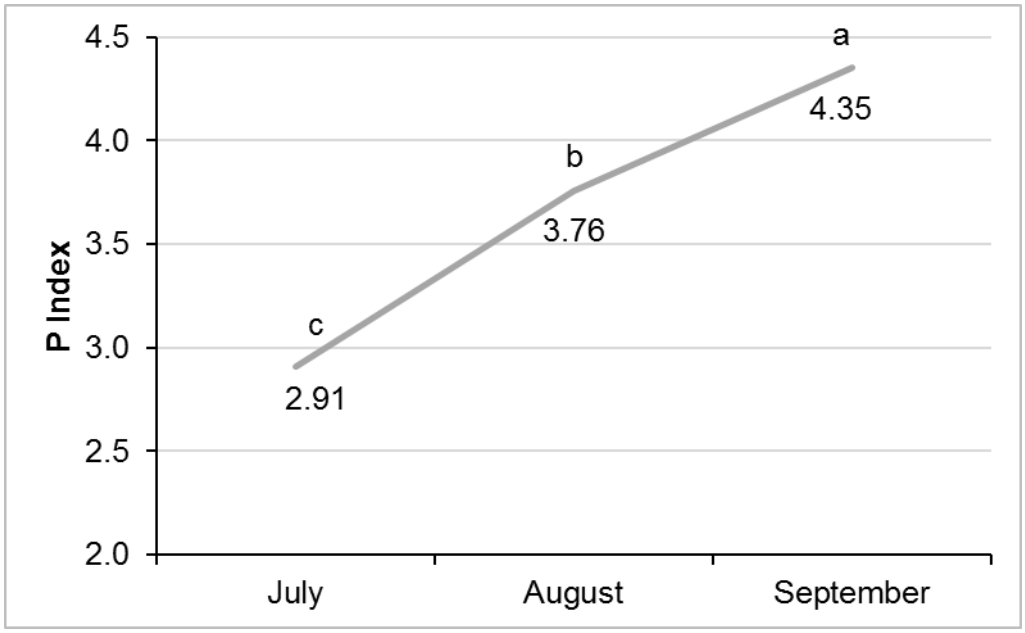

Figure 3.20 Performance index (P Index) variation on strawberry leaves of plants during the experimental period of summer 2018. Means followed by the same letter are not significantly different (P≤ 0.05) (n=72).

3.2.2.2 Chlorophyll content

Our results indicated that there is no significant difference between biostimulant treatments for the leaf chlorophyll content (Table 3.12). However, the time of measurement showed a significant difference (*P*=0.014). As shows in the Figure 3.21, chlorophyll content increased steadily from July to September. The highest value of chlorophyll content was observed in September with a mean value of 40.9 SPAD unit.

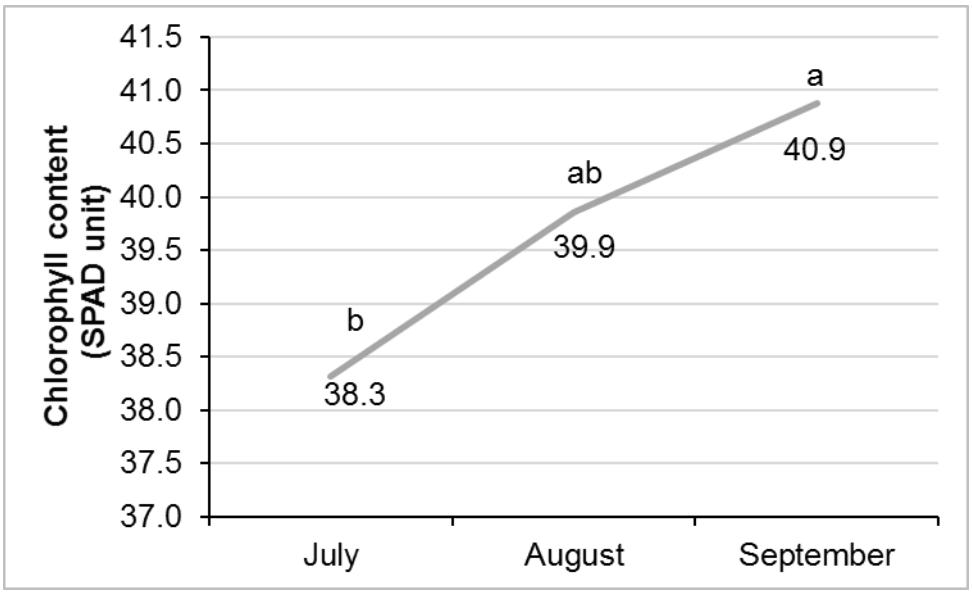

Figure 3.21 Evaluation of the chlorophyll content on strawberry leaves during the experimental period of summer 2018. Means followed by the same letter are not significantly different (P≤ 0.05) (n=72).

3.2.3 Photosynthesis

Figure 3.22 shows the light saturation curves related to each treatment. Biostimulant treatments had no positive effects on leaf photosynthetic rate under low PPFD. On the other hand, at higher PPFD of 500 µmol $m^{-2}s^{-1}$, treatment with biostimulants increased photosynthetic rates compared with control plants, except for MYC+BACT, which was similar to control plants. However, this increase was not significant at P<0.05 due to large variation.

Photosynthesis parameters resulting of the light saturation curves are shown in Table 3.13. The results showed that treatments with biostimulants did not influence the maximum photosynthetic rate (Amax) and dark respiration rate (Rd), while the maximum quantum yield (Φ), which represents the initial slope of the light-response curve of the net

photosynthetic rate was significantly lower (-30%) in the plants treated with citric acid compared with control plants. The other treatments were not significantly different from the control.

Table 3.13 Photosynthesis parameters of strawberry leaves with studied biostimulants during summer experiment (n=4).

Treatments	**Maximum rate of photosynthesis (μmol CO_2 m^{-2} s^{-1})**	**Respiration rate in the dark (Rd) (μmol CO_2 m^{-2} s^{-1})**	**Maximum quantum yield (Φ)**
CONTROL[z]	17.30	-1.548	0.081a
MYC	16.94	-1.085	0.075a
MYC+BACT	14.02	-1.528	0.081a
BACT	18.58	-1.376	0.082a
CITRIC	16.66	-1.174	0.057b*
CITRIC+LACTIC	18.83	-1.108	0.071ab
P values			
Biostimulants	0.250	0.669	0.014

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC = Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).

[x]means of the same column with different letters are significantly different at $P<0.05$.

*Treatments are different from their respective control.

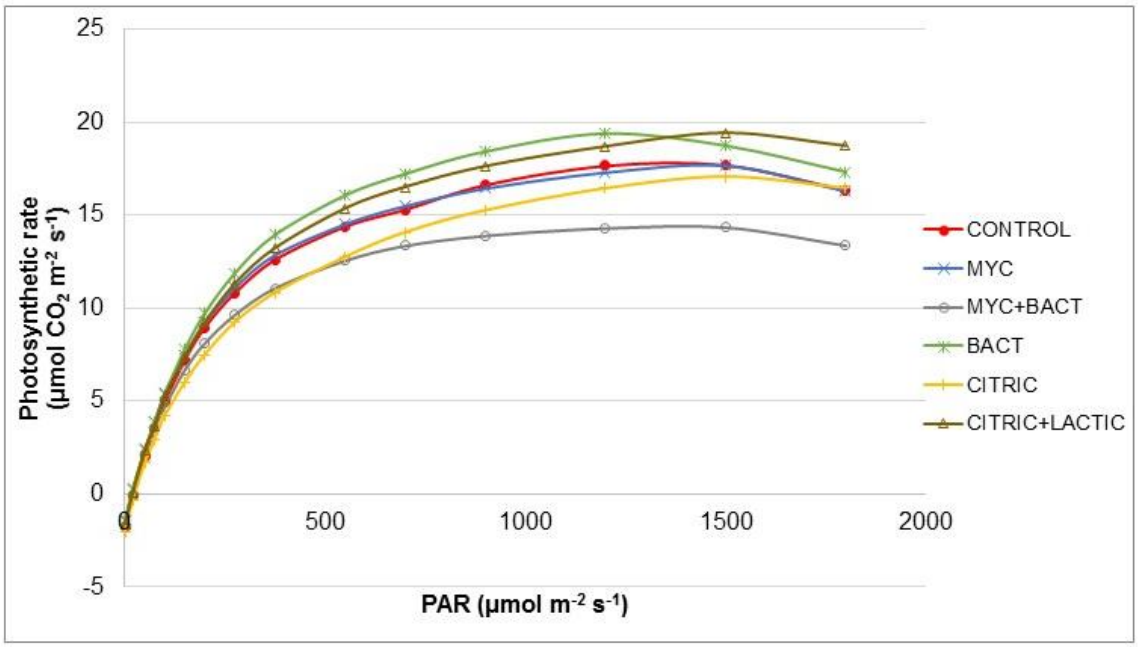

Figure 3.22 Light saturation curves of strawberry leaves cultivated in the high tunnel (n=4).

3.2.4 Non-destructive growth parameters

Results presented in Table 3.14 show that growth parameters such as the number of flowering stalks, number of crowns, and diameter of crowns (mm) were significantly influenced by time of measurement and by the biostimulant treatments, while the number of leaves was not significantly influenced by the biostimulants and time. No interaction was observed between biostimulant treatments and time.

The number of flowering stalks was increased for plants treated with a mixture of mycorrhiza and bacteria (+25%), citric acid (+31%), and citric + lactic acid (+43%) compared with the control. Similarly, MYC+BACT as well as citric + lactic acid increased the number of crowns by 14% compared with the control. The crown diameter was increased for plants treated with the combination of bacteria (+12%) and with citric+ lactic acids (+16%).

The effect of time was observed for the number of flowering stalks (P=0.026), number of crowns (P<0.001), and the diameter of crowns (mm) (P=0.026) parameters. These growth parameters increased significantly from July to September. The highest value was observed in September for the number of flowering stalks (mean value of 8.97), the

number of crowns (mean value of 4.92), and the diameter of crowns (mean value of 49.11 mm). Also, all growth parameters showed the lowest value in July.

Table 3.14 Influence of the biostimulant treatments on the growth parameters of strawberry plants grown under high-tunnel. Data are means of three measurements (July, August, and September) during summer 2018 (n=36).

Treatments		Number of leaves	Number of flowering stalks	Number of crowns	Diameter of crowns (mm)
CONTROL[z]		21.14	6.53 d[x]	4.08 b	44.15 c
MYC		20.44	7.14 cd	3.92 b	48.14 abc
MYC+BACT		20.64	8.14 abc*	4.67 a*	44.50 c
BACT		21.86	7.83 bcd	4.31 ab	49.48 ab*
CITRIC		20.44	8.58 ab*	3.94 b	45.83 bc
CITRIC+LACTIC		23.08	9.33 a*	4.67 a*	51.18 a*
Time	July	19.69	6.60 b	3.43 b	44.65 b
	August	21.14	8.21 ab	4.44 a	47.89 ab
	September	22.97	8.97 a	4.92 a	49.11 a
P values					
Biostimulant (B)		0.640	0.001	0.019	0.013
Time (T)		0.416	0.026	<0.001	0.026
B × T		1.000	1.000	0.914	1.000

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC = Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).
[x]means of the same column with different letters are significantly different at $P<0.05$.
*Treatments are different from their respective control.

3.2.4.1 Destructive growth parameters

3.2.4.2 Shoot fresh and dry biomass

Shoot biomass was significantly influenced by the biostimulant treatments. Results presented in the Table 3.15 indicated that shoot fresh and dry biomass of treated plants increased in average by 52% and 55%, respectively, compared to the control plants, except for the fresh biomass of the combination of bacteria which was not different from the control.

Table 3.15 Influence of the biostimulant treatments on fresh and dry aerial biomass of strawberry plants grown under high-tunnel. Data are collected at the end of the experiment in summer 2018 (n=12).

Treatments	Fresh shoot biomass per plant (g)	Dry shoot biomass per plant (g)
CONTROL[z]	83.91 b	18.47 b
MYC	131.48 a*	30.38 a*
MYC+BACT	120.68 a*	28.26 a*
BACT	109.28 ab	24.41 a*
CITRIC	133.63 a*	31.05 a*
CITRIC+LACTIC	124.33 a*	29.48 a*
P values		
Biostimulant	0.038	0.001

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC = Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).
[x]means of the same column with different letters are significantly different at $P<0.05$.
*Treatments are different from their respective control (LSD).

3.2.5 Mineral analysis of leaves

For all treatments, leaf mineral concentrations in N, P, K, Ca, and Mg were not significantly different between biostimulant treatments (Table 3.16). However, the time of sampling showed a significant difference for N and P concentrations, where their leaf concentration increased from July to August by 20% for N and 38% for P.

Table 3.16 Influence of the biostimulant treatments on mineral concentration (%) in the leaves of strawberry plants grown under high-tunnel. Data are means of three measurements during summer 2018 (n=8).

Treatments		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
CONTROL[z]		1.86[x]	0.480	2.05	1.20	0.293	157.56 a
MYC		1.94	0.446	2.09	1.15	0.284	140.85 ab
MYC+BACT		2.09	0.440	2.03	1.07	0.285	126.90 bc
BACT		1.99	0.500	1.92	1.19	0.316	110.55 c
CITRIC		2.11	0.438	2.17	1.10	0.318	101.74 c
CITRIC+LACTIC		2.14	0.483	2.04	1.22	0.311	118.24 bc
Time	July	1.84 b	0.390 b	1.98	1.203	0.307	144 a
	August	2.20 a	0.539 a	2.13	1.103	0.295	107.95 b
P values							
Biostimulant (B)		0.246	0.308	0.539	0.817	0.536	0.005
Time (T)		<0.001	<0.001	*0.086*	0.135	0.452	0.001
B × T		0.215	0.386	0.180	0.947	0.879	0.309

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC = Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).
[x]means of the same column with different letters are significantly different at *P*<0.05.
*Treatments are different from their respective control.

3.2.6 Yield

Our results indicated that total and marketable yield parameters were significantly influenced by the biostimulant treatments and the date of harvest (Table 3.17). No significant interactions were observed between biostimulant treatments and time of harvest.

Although some differences were observed between the biostimulant treatments, no significant difference between any biostimulant and the control was observed. Plants grown by a mixture of mycorrhiza and bacteria (MYC+BACT), however, produced a lower total (-12%) and marketable (-13%) weight of fruits compared to the other biostimulant treatments. No significant treatment effect was observed for the number of unmarketable fruits.

As expected, a time effect was observed for all yield parameters (see annex B). Yield parameters were higher in August (between 14 and 17 weeks after plantation) with the highest value for the total number and weight of fruits observed on week 16 after plantation (a mean of 7.34 fruits per plant and 78.58 g per plant). Similar results were observed for marketable and unmarketable fruits.

Table 3.17 Influence of the biostimulant treatments on yield parameters of strawberry plants grown under high-tunnel. Data are means of three measurements during summer 2018 (n=158).

Treatments	**Total number of fruits/ plant/weeks**	**Total weight of fruits/ plant/weeks (g)**	**Number of marketable fruits/ plant/weeks**	**Weight of marketable fruits/ plant/weeks (g)**	**Number of unmarketable fruits/ plant/weeks**	**Weight of unmarketable fruits/ plant/weeks (g)**
CONTROL[z]	3.56 ab[x]	39.04 ab	2.77 ab	34.30 ab	0.794	4.739
MYC	3.85 a	43.01 a	2.98 a	37.56 a	0.875	5.455
MYC+BACT	3.36 b	37.26 b	2.56 bc	32.55 b	0.801	4.712
BACT	3.68 ab	41.50 a	2.88 a	36.59 a	0.818	4.908
CITRIC	3.90 a	42.89 a	2.96 a	37.08 a	0.940	5.809
CITRIC+LACTIC	3.81 a	42.57 a	2.99 a	37.68 a	0.814	4.897
P values						
Biostimulant (B)	0.027	0.029	0.012	0.039	0.388	0.311
Time (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
B × T	0.750	0.536	0.693	0.583	0.533	0.751

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC = Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).

[x]means of the same column with different letters are significantly different at $P<0.05$.

*Treatments are different from their respective control.

3.2.7 Fruit quality

3.2.7.1 Total sugar level (°Brix)

No significant difference in the total sugar level (brix) of fruits treated with biostimulants was observed (Table 3.18). However, the time of measurement showed a significant difference (*P*<0.001), as shown in Figure 3.23, with the highest brix in mid-July.

Table 3.18 Influence of the biostimulant treatments on fruit quality parameters of strawberry plants grown under high-tunnel. Fruits sampled in July, August, and September 2018 (n=24).

Treatments		Total sugar level (°Brix)	Total polyphenols (mg/100g DW)	Anthocyanins (mg/100gDW)
CONTROL[z]		8.46	6610	303.7b
MYC		8.37	7066	407.6a*
MYC+BACT		8.60	6812	437.3a*
BACT		8.48	6839	474.0a*
CITRIC		8.75	6610	466.9a*
CITRIC+LACTIC		8.60	6916	442.6a*
Time	July	8.79a	5780c	272.9b
	August	8.38b	8032a	281.6b
	September	8.42b	6615b	711.5a
P values				
Biostimulant (B)		0.573	0.318	0.001
Time (T)		<0.001	<.0001	0.001
B × T		0.786	0.604	0.002

[z]CONTROL= without biostimulant; MYC= mycorrhiza (*Rhizoglomus irregulare)*; BACT= three strains of bacteria (*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens)*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC = Citric acid (citric acid-based formulation., AEF GLOBAL Inc.); CITRIC+LACTIC= Citric and lactic acid (citric and lactic acids based formulation).

[x]means of the same column with different letters are significantly different at *P*<0.05.

*Treatments are different from their respective control.

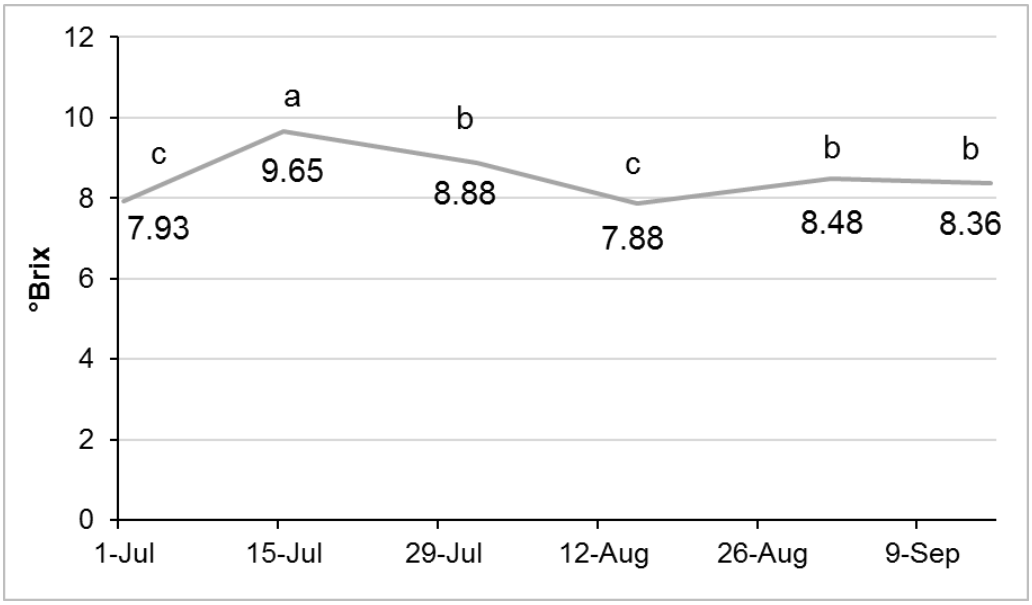

Figure 3.23 Effect of time on the total sugar level (°Brix) of strawberry plants. Means followed by the same letter are not significantly different (P ≤ 0.05) (n=5).

3.2.7.2 Total phenolics assay and anthocyanins

The concentration of polyphenols in the fruits were not influenced by the biostimulant treatments (Table 3.18). However, the time of measurement has significantly influenced the fruit polyphenol concentration (*P*<0.0001) (Figure 3.24). No interaction was observed between treatments and time.

The highest value of polyphenols was observed in August (8032 mg/100DW), followed by fruits sampled in September (6614 mg/100gDW).

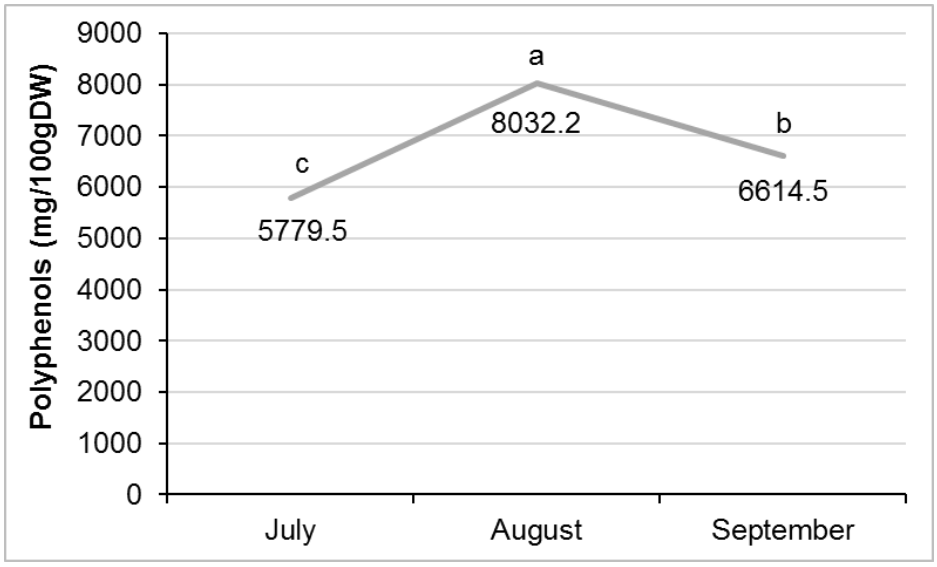

Figure 3.24 Effects of time on the total polyphenol concentration of strawberry fruits. Means followed by the same letter are not significantly different (P ≤ 0.05) (n=12).

For the anthocyanin berry content, a significant difference was observed between treatments (*P*= 0.0009) and time of measurement (*P*= 0.0011). However, a significant interaction was observed between treatments and time (*P*= 0.0021) (Table 3.18).

The interaction between the time and treatments is presented in the Figure 3.25. The influence of the biostimulants was observed only in the September where, all treatments increased (+74% to +137%) the concentration of the anthocyanins compared to the control. Moreover, no difference was observed between the sampling dated for the control plants.

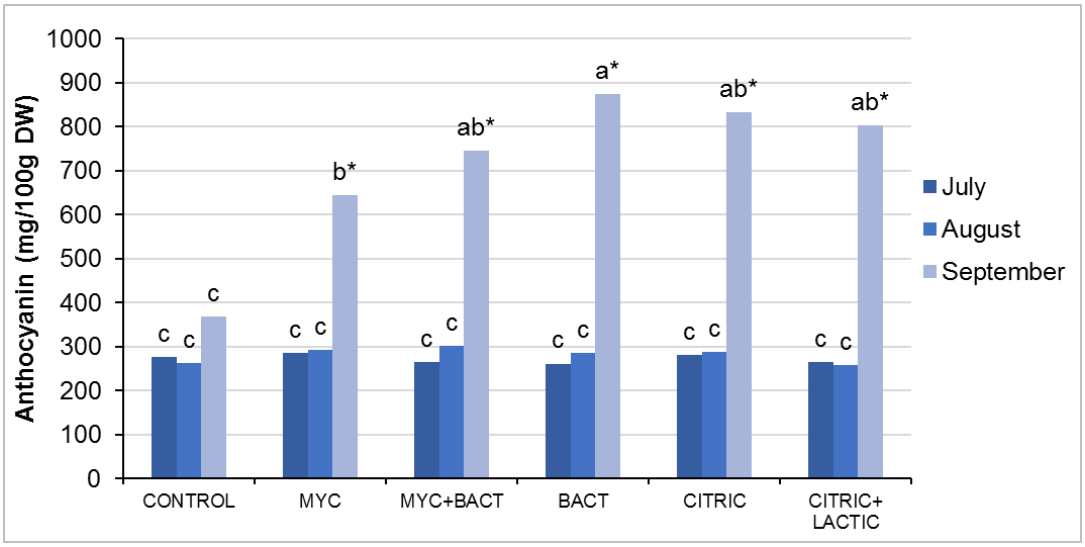

Figure 3.25 Effects of studied biostimulants on anthocyanin concentration of the fruits. Means followed by the same letter are not significantly different (P ≤ 0.05) (n=12).

3.2.8 Principal component analysis (PCA)

A principal component analysis (PCA) was conducted with some important variables and the biostimulant treatments. PC1 and PC2 explained 58.41% of the total variance, with accounting 31.04% and 27.37%, respectively (Figure 3.26). The treatments with citric acid (E1), citric and lactic acid (E2), and bacteria (D) are located in the right two quadrats, while the treatments with mycorrhiza (B), combination of mycorrhiza and bacteria (C), and control (A) are in the two left quadrats. The main variables associated with PC1 are the marketable yield, FDA, SPAD, Mg, P and Ca, while PC2 was mainly related to leaf N content. No clear clustering was observed between the biostimulants and the control. The combination of bacteria was closely associated with the marketable yield, while the mixture of mycorrhiza and bacteria was associated with soil FDA. Citric acid treatment was closely associated with fruit anthocyanins, and citric and lactic acids with leaf Mg content. The control treatment was not associated with any of these variables that explain more than 58% of the observed variations.

Similarly, to the greenhouse experiment, FDA was inversely related to leaf P and Ca. However, in contrast to the greenhouse experiment, SPAD values were inversely associated with leaf Mg, and not related to N. It was also inversely related to the

marketable yield. Fruit anthocyanin content was associated to leaf N and Mg content, but inversely related to P and Ca.

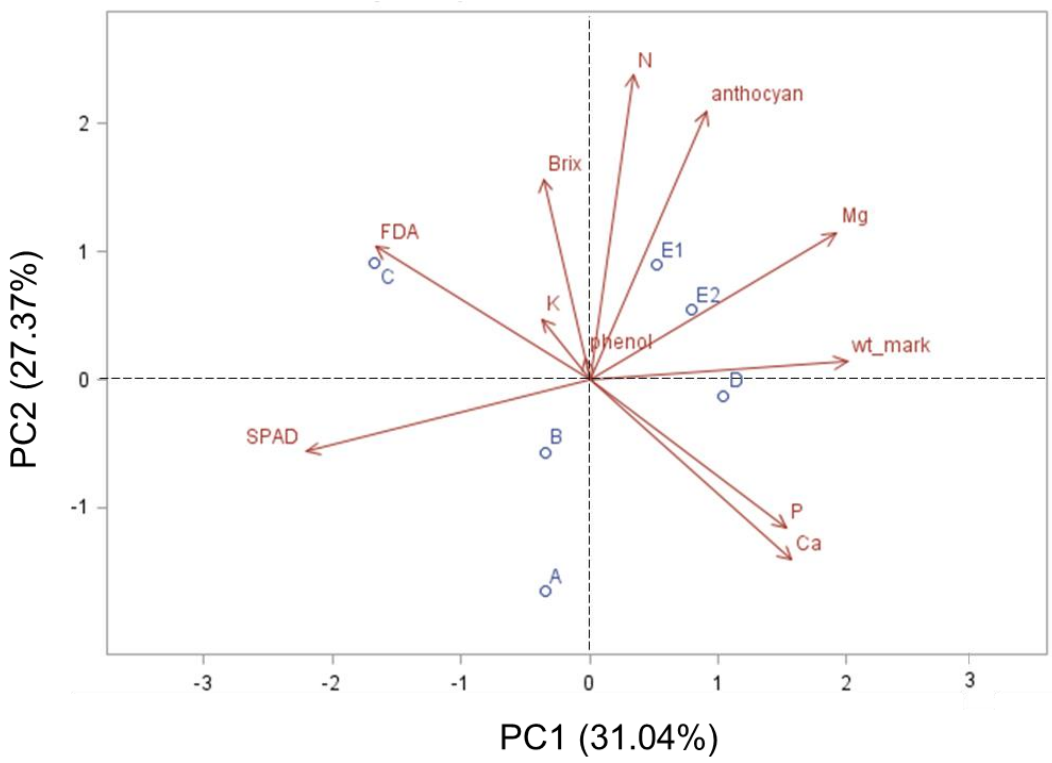

Figure 3.26 Relationships between leaf mineral content, physiological, yield, quality, and soil activity parameters during the greenhouse experiment (PCA). A: control, B: mycorrhiza, C: combination of mycorrhiza and bacteria, D: bacteria. E1: citric acid. E2: citric and lactic acids.

3.2.9 Relationship between soil activity and leaf mineral content, and the physiological, growth, yield, and quality parameters

We have observed no correlations between FDA and leaf mineral contents, physiological, growth, and quality parameters (Tables 3.19 and 3.20). However, negative correlations were observed between the FDA and the total fruit number (r= -0.873; P=0.010) and weight (r=-0.851; P=0.015) as well as the marketable fruit number (r=-0.901; P=0.006) and weight (r=-0.843; P=0.017).

Negative correlation was observed between chlorophyll content (SPAD) with concentration of Mg (r= -0.971; *P*=0.000) and Zn (r= -0.958; *P*=0.001) in the leaves. Positive correlation was observed between maximum rate of photosynthesis (Amax) with

P (r= 0.779; *P*=0.040) and Ca (r= 0.884; *P*=0.008), while quantum efficiency (Φ) was negatively correlated to K (r= -0.801; *P*= 0.030). No correlation was observed between leaf mineral content and other physiological parameters (Table 3.19).

The flowering stalk was positively correlated with nitrogen concentration (r= 0.819; *P*=0.024) and number of crowns (r= 0.754; *P*=0.050), while the number of crowns was correlated with Fe (r= 0.835; *P*=0.020). Besides, diameter of crowns was positively correlated with the number of leaves (r= 0.917; *P*= 0.004), number of flowering stalks (r= 0.814; *P*= 0.026), and number of crowns (r= 0.781; *P*= 0.038) (Table 3.20).

In relation to the yield parameters, dark respiration rate (Rd) was positively correlated with the total number of fruits (r= 0.897; *P*= 0.006), number of marketable fruits (r= 0.893; *P*=0.007), and weight of unmarketable fruits (r= 0.918; *P*=0.004). Besides, positive correlation was observed between maximum rate of photosynthesis with total fruit weight (r= 0.763; *P*= 0.046) and marketable fruits (r= 0.759; *P*= 0.048). Number of unmarketable fruits was negatively correlated with quantum efficiency (r= -0.826; *P*= 0.022) (Table 3.20).

Positive correlation was observed between fruit Brix and leaf nitrogen content (r= 0.815; *P*= 0.026), while the fruit anthocyanins was positively correlated with N content (r= 0.760; *P*= 0.048). Besides, negative correlation was observed between Brix and Fv/Fm (r= -0.763; *P*= 0.046), and anthocyanins with leaf concentration of Na (r= -0.932; *P*= 0.002) (Table 3.20).

Table 3.19 Relationships between the mineral content of leaves and soil activity and physiological, growth, yield and quality parameters of strawberry plants in the high tunnel (Summer 2018).

		N	P	K	Ca	Mg	Na	Fe	Zn	B	FDA
Soil activity											
	FDA	0.332	-0.191	-0.305	-0.506	-0.310	-0.028	0.332	-0.387	0.436	1.000
	P value	0.467	0.682	0.506	0.247	0.499	0.952	0.467	0.391	0.328	
Leaf mineral content											
	N	1.000	-0.271	0.202	-0.345	0.460	-0.788	0.413	0.479	-0.261	0.332
	P value		0.556	0.665	0.449	0.299	0.035	0.357	0.277	0.572	0.467
	P	-0.271	1.000	-0.771	0.858	0.412	0.060	-0.038	0.366	0.275	-0.191
	P value	0.556		0.042	0.014	0.358	0.898	0.935	0.419	0.550	0.682
	K	0.202	-0.771	1.000	-0.421	-0.076	-0.037	0.048	0.098	-0.355	-0.305
	P value	0.665	0.042		0.347	0.872	0.937	0.919	0.835	0.434	0.506
	Ca	-0.345	0.858	-0.421	1.000	0.274	0.275	0.173	0.365	0.345	-0.506
	P value	0.449	0.014	0.347		0.552	0.550	0.710	0.421	0.448	0.247
	Mg	0.460	0.412	-0.076	0.274	1.000	-0.778	-0.271	0.954	-0.575	-0.310
	P value	0.299	0.358	0.872	0.552		0.040	0.556	0.001	0.177	0.499
	Na	-0.788	0.060	-0.037	0.275	-0.778	1.000	0.188	-0.664	0.711	-0.028
	P value	0.035	0.898	0.937	0.550	0.040		0.686	0.104	0.073	0.952
	Fe	0.413	-0.038	0.048	0.173	-0.271	0.188	1.000	-0.076	0.684	0.332
	P value	0.357	0.935	0.919	0.710	0.556	0.686		0.872	0.090	0.467
	Zn	0.479	0.366	0.098	0.365	0.954	-0.664	-0.076	1.000	-0.456	-0.387
	P value	0.277	0.419	0.835	0.421	0.001	0.104	0.872		0.304	0.391
	B	-0.261	0.275	-0.355	0.345	-0.575	0.711	0.684	-0.456	1.000	0.436
	P value	0.572	0.550	0.434	0.448	0.177	0.073	0.090	0.304		0.328

	N	P	K	Ca	Mg	Na	Fe	Zn	B	FDA
Physiological parameters										
Fv/Fm	-0.486	0.374	-0.460	0.346	-0.072	0.104	-0.407	-0.220	-0.189	-0.477
P value	0.269	0.408	0.299	0.448	0.878	0.824	0.365	0.636	0.685	0.279
PI	0.101	0.315	-0.246	0.539	-0.094	0.091	0.578	-0.013	0.313	-0.277
P value	0.829	0.492	0.595	0.212	0.841	0.846	0.174	0.977	0.495	0.548
SPAD	-0.335	-0.343	-0.089	-0.289	-0.971	0.682	0.358	-0.958	0.636	0.472
P value	0.462	0.451	0.849	0.530	0.000	0.091	0.431	0.001	0.125	0.285
A_{max}	-0.114	0.776	-0.291	0.884	0.615	-0.139	-0.060	0.655	-0.114	-0.699
P value	0.808	0.040	0.527	0.008	0.142	0.767	0.898	0.110	0.808	0.081
Rd	0.365	-0.174	0.442	0.146	0.302	-0.384	0.152	0.395	-0.468	-0.662
P value	0.420	0.709	0.320	0.755	0.511	0.395	0.744	0.380	0.290	0.105
Φ	-0.551	0.472	-0.801	0.261	-0.511	0.570	0.058	-0.621	0.667	0.398
P value	0.200	0.285	0.030	0.571	0.241	0.182	0.902	0.137	0.102	0.377
Growth parameters										
Number of leaves	0.424	0.521	-0.219	0.658	0.376	-0.154	0.711	0.523	0.372	-0.087
P value	0.343	0.230	0.637	0.108	0.406	0.742	0.073	0.229	0.411	0.853
Number flowering stalks	0.819	0.119	0.094	0.223	0.530	-0.564	0.628	0.648	-0.023	-0.014
P value	0.024	0.800	0.840	0.630	0.221	0.187	0.131	0.116	0.961	0.976
Number of crowns	0.530	0.376	-0.380	0.358	0.130	-0.128	0.835	0.219	0.571	0.399
P value	0.221	0.406	0.401	0.431	0.781	0.784	0.020	0.637	0.180	0.376
Diameter of crowns	0.429	0.506	-0.315	0.609	0.378	-0.284	0.576	0.451	0.180	-0.192
P value	0.337	0.246	0.491	0.147	0.403	0.537	0.176	0.310	0.699	0.680
Shoot fresh biomass	0.678	-0.598	0.474	-0.486	0.141	-0.616	0.134	0.208	0.137	-0.108
P value	0.094	0.157	0.283	0.269	0.764	0.141	0.774	0.654	0.769	0.818
Shoot dry biomass	0.719	-0.602	0.483	-0.478	0.124	-0.600	0.219	0.148	0.139	-0.064
P value	0.069	0.153	0.273	0.278	0.791	0.154	0.636	0.752	0.766	0.892

	N	P	K	Ca	Mg	Na	Fe	Zn	B	FDA
Yield parameters										
Total number of fruits	0.158	-0.027	0.467	0.266	0.509	-0.379	-0.178	0.588	-0.641	-0.873
P value	0.735	0.953	0.290	0.565	0.243	0.402	0.703	0.165	0.121	0.010
Number marketable fruits	0.081	0.189	0.292	0.486	0.516	-0.289	-0.091	0.604	-0.486	-0.901
P value	0.864	0.684	0.525	0.268	0.236	0.530	0.846	0.151	0.269	0.006
Weight of marketable fruits	0.158	0.209	0.202	0.434	0.532	-0.385	-0.094	0.584	-0.529	-0.843
P value	0.735	0.653	0.664	0.330	0.219	0.394	0.841	0.169	0.223	0.017
Number unmarketable fruits	0.264	-0.583	0.755	-0.422	0.327	-0.490	-0.428	0.336	-0.873	-0.514
P value	0.568	0.169	0.050	0.346	0.474	0.264	0.338	0.461	0.010	0.238
Weight of unmarketable fruits	0.189	-0.548	0.745	-0.343	0.282	-0.420	-0.416	0.302	-0.847	-0.604
P value	0.685	0.202	0.054	0.451	0.540	0.348	0.354	0.511	0.016	0.151
Total weight	0.174	0.062	0.324	0.316	0.518	-0.415	-0.160	0.567	-0.621	-0.851
P value	0.710	0.895	0.478	0.491	0.234	0.354	0.732	0.184	0.137	0.015
Quality parameters										
Brix	0.815	-0.310	0.371	-0.430	0.569	-0.718	0.100	0.594	-0.370	0.319
P value	0.026	0.499	0.412	0.335	0.183	0.069	0.832	0.159	0.414	0.485
Total polyphenols	0.040	-0.114	-0.082	0.149	-0.324	0.059	0.339	-0.347	0.131	-0.143
P value	0.932	0.808	0.861	0.750	0.479	0.900	0.457	0.445	0.779	0.760
Anthocyanins	0.760	0.023	-0.235	-0.337	0.560	-0.932	-0.110	0.411	-0.630	0.104
P value	0.048	0.962	0.612	0.460	0.191	0.002	0.815	0.360	0.130	0.825

Table 3.20 Relationships between physiological, growth, yield and quality parameters (Summer 2018).

	Fv/Fm	PI	SPAD	Nb leaves	Nb flowering stalks	Crown nb	Crown diameter	Shoot FW	Shoot DW
Physiological parameters									
Fv/Fm	1.000	0.465	0.076	-0.137	-0.320	-0.271	0.196	0.023	-0.040
P value		0.293	0.871	0.770	0.485	0.556	0.674	0.961	0.932
PI	0.465	1.000	0.164	0.679	0.501	0.572	0.847	0.276	0.300
P value	0.293		0.725	0.094	0.252	0.180	0.016	0.549	0.513
SPAD	0.076	0.164	1.000	-0.285	-0.433	0.040	-0.264	-0.112	-0.089
P value	0.871	0.725		0.535	0.332	0.933	0.567	0.812	0.850
A_{max}	0.425	0.487	-0.635	0.610	0.371	0.214	0.660	-0.164	-0.179
P value	0.342	0.268	0.126	0.146	0.413	0.645	0.107	0.725	0.700
Rd	0.265	0.586	-0.350	0.357	0.563	0.045	0.548	0.755	0.746
P value	0.565	0.167	0.441	0.432	0.188	0.924	0.203	0.050	0.054
Φ	0.404	0.177	0.610	-0.065	-0.472	0.184	-0.029	-0.625	-0.625
P value	0.369	0.703	0.146	0.890	0.285	0.692	0.951	0.133	0.133
Growth parameters									
Number of leaves	-0.137	0.679	-0.285	1.000	0.844	0.871	0.917	0.030	0.089
P value	0.770	0.094	0.535		0.017	0.011	0.004	0.949	0.850
Number flowering stalks	-0.320	0.501	-0.433	0.844	1.000	0.754	0.814	0.484	0.537
P value	0.485	0.252	0.332	0.017		0.050	0.026	0.271	0.214
Number of crowns	-0.271	0.572	0.040	0.871	0.754	1.000	0.781	0.018	0.091
P value	0.556	0.180	0.933	0.011	0.050		0.038	0.970	0.847
Diameter of crowns	0.196	0.847	-0.264	0.917	0.814	0.781	1.000	0.252	0.287
P value	0.674	0.016	0.567	0.004	0.026	0.038		0.586	0.533
Shoot fresh biomass	0.023	0.276	-0.112	0.030	0.484	0.018	0.252	1.000	0.996
P value	0.961	0.549	0.812	0.949	0.271	0.970	0.586		<.0001
Shoot dry biomass	-0.040	0.300	-0.089	0.089	0.537	0.091	0.287	0.996	1.000
P value	0.932	0.513	0.850	0.850	0.214	0.847	0.533	<.0001	

	Fv/Fm	PI	SPAD	Nb leaves	Nb flowering stalks	Crown nb	Crown diameter	Shoot FW	Shoot DW
Yield parameters									
Total number of fruits	0.277	0.317	-0.621	0.221	0.382	-0.218	0.359	0.521	0.491
P value	0.548	0.488	0.137	0.634	0.397	0.639	0.429	0.230	0.263
Number marketable fruits	0.367	0.471	-0.610	0.384	0.425	-0.076	0.516	0.386	0.361
P value	0.418	0.286	0.146	0.395	0.342	0.872	0.236	0.393	0.427
Weight of marketable fruits	0.416	0.528	-0.593	0.386	0.463	-0.033	0.573	0.479	0.451
P value	0.353	0.223	0.160	0.393	0.296	0.943	0.179	0.277	0.310
Number unmarketable fruits	-0.024	-0.250	-0.455	-0.349	0.074	-0.586	-0.234	0.673	0.631
P value	0.959	0.589	0.305	0.443	0.874	0.167	0.614	0.098	0.129
Weight unmarketable fruits	0.074	-0.153	-0.421	-0.325	0.057	-0.597	-0.187	0.669	0.625
P value	0.875	0.744	0.347	0.478	0.903	0.157	0.689	0.100	0.133
Total weight	0.399	0.433	-0.598	0.277	0.415	-0.141	0.466	0.544	0.511
P value	0.375	0.332	0.157	0.548	0.354	0.763	0.292	0.207	0.241
Quality parameters									
Brix	-0.763	-0.438	-0.523	0.145	0.544	0.207	-0.006	0.363	0.393
P value	0.046	0.326	0.228	0.756	0.207	0.656	0.990	0.423	0.383
Total polyphenols	0.647	0.862	0.404	0.118	0.516	0.175	0.355	0.736	0.718
P value	0.116	0.013	0.369	0.801	0.236	0.707	0.435	0.059	0.069
Anthocyanins	0.110	0.119	-0.427	0.256	0.203	0.280	0.557	0.460	0.457
P value	0.815	0.800	0.339	0.579	0.663	0.543	0.194	0.299	0.302

Table 3.21 (continuity) Relationships between physiological, growth, yield and quality parameters (Summer 2018).

	Total fruit nb	Mark. fruit nb	Mark. Fruit weight	Unmark. fruit nb	Umark fruit weight	Total fruit weight	Brix	Total polyphenols	Anto-cyanins
Physiological parameters									
Fv/Fm	0.277	0.399	0.367	0.416	-0.024	0.074	-0.763	0.647	0.110
P value	0.548	0.375	0.418	0.353	0.959	0.875	0.046	0.116	0.815
PI	0.317	0.433	0.471	0.528	-0.250	-0.153	-0.438	0.862	0.119
P value	0.488	0.332	0.286	0.223	0.589	0.744	0.326	0.013	0.800
SPAD	-0.621	-0.598	-0.610	-0.593	-0.455	-0.421	-0.523	0.404	-0.427
P value	0.137	0.157	0.146	0.160	0.305	0.347	0.228	0.369	0.339
A_{max}	0.602	0.763	0.759	-0.035	0.032	0.656	-0.206	0.046	0.148
P value	0.153	0.046	0.048	0.941	0.946	0.109	0.657	0.922	0.752
Rd	0.897	0.872	0.893	0.625	0.687	0.918	0.052	0.435	0.523
P value	0.006	0.011	0.007	0.134	0.088	0.004	0.913	0.329	0.229
Φ	-0.714	-0.562	-0.540	-0.860	-0.826	-0.616	-0.673	-0.384	0.267
P value	0.071	0.189	0.211	0.013	0.022	0.141	0.098	0.395	0.563
Growth parameters									
Number of leaves	0.221	0.277	0.384	0.386	-0.349	-0.325	0.145	0.256	0.118
P value	0.634	0.548	0.395	0.393	0.443	0.478	0.756	0.579	0.801
Number of flowering stalks	0.382	0.415	0.425	0.463	0.074	0.057	0.544	0.203	0.516
P value	0.397	0.354	0.342	0.296	0.874	0.903	0.207	0.663	0.236
Number of crowns	-0.218	-0.141	-0.076	-0.033	-0.586	-0.597	0.207	0.280	0.175
P value	0.639	0.763	0.872	0.943	0.167	0.157	0.656	0.543	0.707
Diameter of crowns	0.359	0.466	0.516	0.573	-0.234	-0.187	-0.006	0.557	0.355
P value	0.429	0.292	0.236	0.179	0.614	0.689	0.990	0.194	0.435
Shoot fresh biomass	0.521	0.544	0.386	0.479	0.673	0.669	0.363	0.460	0.736
P value	0.230	0.207	0.393	0.277	0.098	0.100	0.423	0.299	0.059
Shoot dry biomass	0.491	0.511	0.361	0.451	0.631	0.625	0.393	0.457	0.718
P value	0.263	0.241	0.427	0.310	0.129	0.133	0.383	0.302	0.069

	Total fruit nb	Mark. fruit nb	Mark. Fruit weight	Unmark. fruit nb	Umark fruit weight	Total fruit weight	Brix	Total polyphenols	Anto-cyanins
Yield parameters									
Total number of fruits	1.000	0.983	0.969	0.955	0.734	0.790	0.070	0.200	0.306
P value		<.0001	0.000	0.001	0.060	0.034	0.881	0.668	0.505
Number marketable fruits	0.969	0.977	1.000	0.985	0.545	0.618	-0.060	0.279	0.229
P value	0.000	0.000		<.0001	0.206	0.139	0.899	0.545	0.622
Weight of marketable fruits	0.955	0.989	0.985	1.000	0.540	0.611	-0.055	0.385	0.369
P value	0.001	<.0001	<.0001		0.211	0.145	0.906	0.394	0.416
Number unmarketable fruits	0.734	0.658	0.545	0.540	1.000	0.991	0.373	-0.109	0.402
P value	0.060	0.109	0.206	0.211		<.0001	0.410	0.815	0.372
Weight unmarketable fruits	0.790	0.722	0.618	0.611	0.991	1.000	0.258	-0.017	0.355
P value	0.034	0.067	0.139	0.145	<.0001		0.576	0.971	0.435
Total weight	0.983	1.000	0.977	0.989	0.658	0.722	0.000	0.333	0.389
P value	<.0001		0.000	<.0001	0.109	0.067	1.000	0.465	0.388
Quality parameters									
Brix	0.070	0.000	-0.060	-0.055	0.373	0.258	1.000	-0.538	0.509
P value	0.881	1.000	0.899	0.906	0.410	0.576		0.213	0.243
Total polyphenols	0.200	0.333	0.279	0.385	-0.109	-0.017	-0.538	1.000	0.265
P value	0.668	0.465	0.545	0.394	0.815	0.971	0.213		0.566
Anthocyanins	0.306	0.389	0.229	0.369	0.402	0.355	0.509	0.265	1.000
P value	0.505	0.388	0.622	0.416	0.372	0.435	0.243	0.566	

4 DISCUSSION

4.1 EFFECT OF BIOSTIMULANTS ON SOIL MICROBIOTA

In general, biostimulant treatments had little effect on the microbial activity, expressed by the hydrolysis of the FDA, and the microbial and abundance and diversity of greenhouse treated plants. However, under conventional management the soil microbial activity of plants treated with bacteria was higher than the control (Figure 3.4b). The relative abundance of fungi also showed some differences (Figure 3.6). In contrast with our results, many researchers reported beneficial effects of seaweed extract, *Trichoderma* and citric acid on the microbial activity and their population in the soil. For example, Khan et al. [144] reported the enhancement in the growth of beneficial soil microbes due to the use of the seaweed extracts. The reason for increasing the number and activity of the microorganism may be related to the soil structure (physical, chemical, and biological properties) and improvement of the moisture-holding capacity of the soils treated with seaweed extracts. In our study, a peat-based growing media having optimal physico-chemical properties was used, which may explain these differences. Furthermore, Alam et al. [157] and Spinelli et al. [289] reported beneficial influence of the seaweed extract on their bacterial population and microbial activity.

In the study of Zhang et al. [290] *Trichoderma* inoculation or combination of *Trichoderma* with ferrihydrite improved microbial diversity of the soil. Besides, Hosseini et al. [291] reported that citric acid improved the activity of the soil microorganisms. This effect could be related to the positive impact of citric acid on the mobility of the phosphorous in the soil [292]. However, in our study, we did not observe any effect of biostimulants on leaf mineral concentration compared with the control. On the other hand, most of the biostimulants increased soil CO_2 efflux (Figures 3.7 and 3.8) in both growing systems compared with their respective control, which suggest higher soil and root activity.

In the high tunnel experiment, a higher microbial activity was observed in the treatment with combination of mycorrhiza and bacteria. Other treatments did not show significant difference with control (Figure 3.18). In agreement with our results, Kim et al. [293] also observed a higher soil microbial activity after the inoculation with a combination of bacteria (*Enterobacter agglomerans*) and vesicular-arbuscular mycorrhizae (*Glomus Etunicatum*).

4.2 EFFECT OF BIOSTIMULANTS ON PLANT GROWTH AND DEVELOPMENT

4.2.1 Photosynthetic performance parameters

For both experiments, our investigations on strawberry plants showed that biostimulants did not increase the physiological parameters when expressed as Fv\Fm, PI, SPAD, Amax, Rd and Φ (Table 3.1). For the performance index, although not significant, our greenhouse study showed that *Trichoderma* outperformed the control (without biostimulant) by 10% when conventionally cultivated. On the other hand, the chlorophyll content (SPAD) of the organic treated plants with the bacteria treatment was negatively affected, while the maximum quantum yield of the high tunnel plants was reduced by 30% compared with the control (Table 3.13).

Although the benefice of using biostimulants on the photosynthetic performance was not observed in the present study, several studies reported the increase of the chlorophyll content by using seaweed extracts [294]. According to Spinelli et al. [289] and Fan et al. [295], seaweed extract contains betaine compounds and cytokinin-like activity which have direct effect on the biosynthesis of chlorophyll. Karlidag et al. [296] reported that Bacillus increased the chlorophyll content of strawberry leaves submitted to salt stress. Zare-maivan et al. [297] reported the similar positive effects on chlorophyll content by using mycorrhiza (Vesicular-Arbuscular Mycorrhiza) on maize. Nitrogen-fixing bacteria significantly increased chlorophyll content and uptake of macro- and micronutrients in tomato and red pepper [172]. Although several studies reported that biostimulants enhance mineral availability and promote plant nutrient uptake [297], among all treatments, a mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria with low fertilization in the organic growing system showed the lowest P Index and chlorophyll content compared to the control and other treatments. However, this low fertilizer treatment did not impact Fv/Fm, maximum photosynthesis rate (Amax), dark respiration rate (Rd) and the maximum quantum yield (Φ).

4.2.2 Non-destructive growth parameters

In greenhouse experiments, biostimulant treatments did not significantly increase growth parameters. On the other hand, plants under low fertilization had lowest growth parameters compared with respective control (Table 3.3). In contrast to our expectation, conventionally grown plants treated with combination of mycorrhiza and bacteria reduced the number of flowering stalks compared with its respective control. Neverthless, although not significant, the number of leaves and the number of flowering fruits stalks increases by 9% and 13% by using citric acid, respectively.

Under high tunnels, however, some biostimulant treatments outperformed the control treatment in terms of the growth parameters except for the number of leaves, where no significant effect was observed. The citric and lactic acids treatment, which was not studied in the greenhouse experiments, gained better non-destructive growth parameters compared to the control, followed by citric acid, as well as a mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria (Table 3.4). In agreement with our results, Talebi et al. [298] and Hajireza et al. [234] reported an increase in the number of flowers and the diameter of flowers for ornamental plants (*Rosa hybrida* L. and *Gazania rigens* L.) by spraying organic acids such as citric acid, malic acid, and salicylic acid. Besides, several studies reported the positive effects of citric acid to increase plant height on dill [229], stem diameter, and number of leaves on maize [299]. Addition to these studies, Backer et al. [171] reported the effect of plant growth-promoting rhizobacteria to the enhancement of plant growth. According to El-Yazal et al. [299], citric acid has antioxidant effect and this antioxidant effect could be improving cell division and protect plant cells against free radicals.

The increase of growth parameters by citric acid might be related to the improvement of the mineral nutrition such as phosphorous and calcium [300, 301]. Although no significant effect was observed for leaf P and Ca content, the PCA (Figure 3.17) showed a close relationship between leaf P and Ca content and the citric acid treated plants. In addition, we think that exogenous use of citric acid may increase internal citric acid, which is involved in the Krebs cycle [302] and increase the biosynthesis of metabolites, resulting in improved relative growth rate and photosynthesis performance [303]. In contrast with our results, several studies showed significant positive effects of seaweed extract [120, 289] on strawberry plants cv Queen Elisa. It was also reported that growth parameters of vegetable crops were improved by using *Trichoderma* [221], beneficial bacteria [11] and mycorrhiza [207, 302].

4.3 EFFECT OF BIOSTIMULANTS ON PLANT BIOMASS AND LEAF AREA

For the greenhouse experiment, biostimulant treatments did not enhance plant biomass and leaf area, except for root biomass of organic plants treated with citric acid. In contrast, conventionally grown plants treated with citric acid had lower root biomass and plants treated with seaweed extract had the lowest shoot dry biomass, root fresh and dry biomass and leaf area compared with the conventional control. Although not significant with control, treatment with a mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria in conventional management had higher fresh and dry biomass compared to the other treatment. On the other hand, all biostimulant treatments in the high tunnel experiment

increased by 52 and 55% their shoot fresh and dry biomass, respectively, compared with the control. Like other parameters, low fertilization treatment produced lowest fresh and dry biomass compared with control and other treatments.

Our high tunnel results are in agreement with Xie et al. [304] who reported that mycorrhiza (*Rhizophagus irregularis*) and bacteria (*Bacillus amyloliquefaciens*) increased plant biomass of *Trifolium repens* and *Fragaria vesca*. Similar results were also observed by Toro et al. [305] for onion plants. Their results showed that inoculation with *Glomus intraradices* and *Bacillus subtilis* significantly increased plant biomass. Ahmed and Shalaby [306] reported that using seaweed extract with compost increased plant fresh and dry biomass and leaf area compared with other treatments.

4.4 EFFECT OF BIOSTIMULANTS ON MINERAL CONTENT OF LEAVES

In contrast to our expectation, biostimulant treatments did not improve leaf nutrient content, and did decrease leaf N content of organic plants treated with citric acid (Tables 3.5, 3.6). Low fertilization reduced leaf N, P, and K concentrations compared with control. Like the greenhouse experiment, in high-tunnel, biostimulant treatments had no significant effect on leaf mineral concentration. However, according to the PCA of the greenhouse experiment, the leaf P content of conventionally grown plants was associated to citric acid and mycorrhiza, while Ca leaf content was related with P plants treated with seaweed extract (Figure 3.17). For organically-grown plants, leaf N content was related to plants treated with mycorrhiza and bacteria, while leaf $N\text{-}NO_3$ content with plants treated with citric acid, and leaf $N\text{-}NH_4$ with plants treated with mycorrhiza, bacteria, and seaweed. On the other hand, organically-grown plants treated with citric acid reduced leaf N concentration, while foliar spray of citric acid increased the accumulation of Ca in the leaves in conventionally grown plants in March. Under high-tunnel, the only relationship observed on the PCA was between the leaf Mg content and the plants treated with lactic and citric acids (Figure 3.26).

In the literature, several studies reported an increase of leaf mineral concentration by using biostimulants [231, 307, 308]. El-Minawy et al. [20] demonstrated the positive effects of seaweed extract on the accumulation of the potassium in the strawberry leaves, which have a similar pattern of results with our findings. Colla et al. [221] reported the higher concentration of the P, Fe, Zn, and B in leaves of the zucchini crops inoculated with *Trichoderma*. Positive effects of nitrogen-fixing bacteria on the nutrient uptake of the plants were reported by authors in different crops [309, 310]. Egamberdiyeva [311] reported

that maize plants treated with bacteria such as *Bacillus* spp. had more efficiency to uptake N, P, and K from the nutrient-deficient calcisol soil. Oliveira et al. [312] reported the high level of N in the maize leaf by inoculation of plants with *A. brasilense.* In addition, inoculation of barley plants with bacteria increased the N concentration in the soil and plant [313]. In research by El-Yazal [299], high concentration of nitrogen, phosphorous and potassium were observed in the maize plants sprayed with combination of citric acid and some micronutrients (Fe, Mn and Zn). These different results may be explained that under protected crops and soilless growing systems, optimal growing conditions and lower abiotic stresses are observed, which might have mitigated the positive impact of biostimulants reported in the literature.

4.5 EFFECT OF BIOSTIMULANTS ON YIELD AND BERRY QUALITY

4.5.1 Yield

For the greenhouse conventionally grown plants, citric acid increased the number (around 20%) and weight (15%) of total and marketable yields compared with the control (Table 3.7), although no significant difference was observed for the number of leaves, flowering fruit stalks and crowns (Table 3.3). To a lesser extent, the marketable yield of organically-grown plants was also increased by using citric acid. Whereas the mixture of mycorrhiza and bacteria increased the marketable yield (+7%) of organically-grown plants, it reduced the number of fruits of conventionally grown plants.

However, the application of biostimulants did not enhance strawberry yield under high-tunnel. In contrast, the mixture of mycorrhiza and bacteria reduced total (-12%) and marketable (-13%) yields (Table 3.7). Although not significant with the control, citric and citric and lactic acids treatments also resulted in an increase of 8 to 10% in both total and marketable yield. This gain of yield could have a direct relationship with the advantage of citric and citric and lactic acids treatments on the flowering fruit stalk parameter (Table 3.3).

Our results are in line with the finding of El-Yazal [299] who reported that citric acid increased yield in terms of number and weight of grain on maize. Besides, Abd-Allah et al. [314] indicated that the application of citric acid increased plant height, yield and protein content of the common bean, pea, and faba bean. Also, the beneficial effects of the citric acid on yield components were reported by Abido et al. [315] on sugar beets and Fawy and Atyia [316] on wheat. The enhancement in the yield may be related to the improving effect of citric acid on growth parameters. However, we did not observe the improvement in the

growth parameters by foliar spray of citric acid. Moreover, little information has been reported about the citric acid mechanism on plant productivity.

4.5.2 Fruit quality

Quality attributes showed significant difference between treatments in both greenhouse and high tunnel experiments. We hypothesized that fruit quality would be higher in the treatments with biostimulants compared with the control. In the greenhouse experiment, conventional treatment with *Trichoderma* and a mixture of mycorrhiza and nitrogen-fixing endosymbiosis bacteria in organic part significantly increased fruit quality parameters such as Brix, or/and total polyphenols, or/and anthocyanins in the fruits. Similarly, in high-tunnel, all biostimulants treatments increased anthocyanin concentration of fruits compared with the control, but no significant improvement was observed for Brix and total polyphenols.

Our results regarding the effect of *Trichoderma*, mycorrhiza, bacteria, and citric acid on fruit quality are in line with several findings. Lingua et al. [212] reported that inoculation with arbuscular mycorrhizal fungi and plant growth-promoting *Pseudomonads* increased anthocyanin concentration of strawberry plants cv Selva under reduced fertilization. In addition, Todeschini et al. [317] showed that inoculation with plant growth promoting bacteria increase sugar and anthocyanin concentrations of strawberry plants cv Elyana. Pascale et al. [318] reported that application of *Trichoderma harzianum* improved total amount of polyphenol and antioxidant activity in grapes. Besides, fruits inoculated with mycorrhiza significantly increased the quantity of glucose content in the tomato plants compared with other treatments [319].

Overall, individual positive effects of biostimulants on biosynthesis of sugar in the different plants were reported by several authors [261, 320]. They mentioned that the higher biosynthesis of sugar could be associated with the higher chlorophyll content, chlorophyll fluorescence, net photosynthesis, and photosystem II efficiency. In our study, biostimulants had no positive impact on these parameters, which may explain why we observed no concluding impact of biostimulants on the fruit Brix index for both experiments.

4.6 DIFFERENCE BETWEEN ORGANIC AND CONVENTIONAL GROWING SYSTEMS

Higher microbial activity and CO_2 efflux were observed in the organic treatment compared to the conventional one (Figures 3.1, 3.8). The abundance of the microorganisms was also higher in the treatments of the organic growing system compared to the conventional one (Figure 3.3). This gain in microbial activity and CO_2 efflux can be explained by the use of

organic manure containing high organic nitrogen, which increases the availability of the organic carbon in the growing medium. Our results agree with several studies which report the positive effects of the organic fertilizers in the microbial activity of the growing medium [321, 322]. In addition, the higher leaf N-NO_3 and N (except for CO_2 efflux) content observed for organically-grown plants were strongly correlated to these soil parameters (Figure 3.17). Unexpectedly, we have observed a negative correlation between soil activity and the leaf P and Ca content.

Two plant physiological parameters, PI and A_{max}, were different between the organic and conventional growing systems. Strawberry plants grown organically had higher PI compared with conventionally grown plants, while its A_{max} was lower. Besides, other physiological parameters such as chlorophyll content and Fv/Fm did not show any significant difference (Table 3.1). The higher PI can be partly explained by the higher leaf N content of organically-grown plants as a strong correlation was observed between PI and leaf N content ($r=0.724$; $p=0.002$). In contrast with our results, several studies reported the enhancement of chlorophyll content of leaves in organic farming. In the study by Macit et al. [323] the chlorophyll content of organically-grown strawberry plants (cv Sweet Charlie) was higher compared with the conventional ones. This could be explained by the fact that under greenhouse environment, all growing parameters are optimized, compared with field experiments.

In terms of growth parameters, organically-grown plants had lower shoot and root fresh and dry biomass as well as the leaf area compared to the conventional ones. These results are in line with findings of Conti et al. [324] who reported conventional farming of strawberries (cv Camarosa) produced higher leaf area compared with organic management. Low plant biomass and leaf area may be explained by the nutrient soil availability and nutrient balance, as nitrogen release and the form of nitrogen differ in both systems. Moreover, this reduced nutrients in the organic growing systems may be related to the mineralization rate of organic manure (Dion et al., 2020) compared with conventional mineral fertilizers. In this study, lower plant biomass and leaf area of organically-grown plants were not related to limited N supply as we observed that leaf N content was higher than conventionally grown plants, and no significant correlation was observed between these growth parameters and the leaf N content. However, P may have limited plant growth of organically-grown plants as their leaf P content was 21% lower than conventionally grown plants. Moreover, leaf P content was strongly correlated with these growth parameters. Similarly to our study, Reganold at al.

[100] analyzed the leaf of the organic and conventionally grown strawberry plants and observed that the concentration of P and K were higher in conventional grown plant than organic ones. In our study, we, however, did not observe important variation of leaf K content.

In accordance with growth parameters, conventional management resulted in higher fruit yield (+10% total yield; +20% marketable yield) compared with organically-grown plants (Table 3.7). Macit et al. [323] reported higher yield of conventionally grown strawberry plants (cv Sweet Charlie) compared with the organic one. Besides, Conti et al. [324] showed that strawberry plants (cv Camarosa) grown in organic farming system produced lower yield (50% less) and lower number of fruits per plant than conventional strawberries. Several studies agree with the yield gap between organic and conventional agriculture. There are several factors to define this gap. The main cause of lower yield of organically-grown strawberries may be related to the limited P availability, which had reduced their growth parameters and their productivity as P was strongly correlated with the total fruit number and weight as well as the marketable fruit number. Indeed, we have observed that all growth parameters, except root biomass, were strongly correlated to total fruit number and weight, while marketable yield was correlated with the number of leaves, flowering stalks, crowns, and crown diameter. On the other hand, the lower marketable yield may also be partly explained by a lower leaf Ca content as a positive correlation between leaf Ca content and marketable yield was observed ($r=0.543$; $p=0.037$). The slightly, but significant, higher unmarketable yield of organically-grown plants may also be related to its higher PI (+5%), which was positively correlated with the unmarketable fruit weight. However, its physiological explanation is not obvious.

No significant difference was observed for fruit quality parameters between both growing systems (Tables 3.8 and 3.9), which agrees with several reviews [9, 94, 325, 326]. However, there are several studies which reported higher fruit quality in organic farming of strawberries compared with conventional. Andrade et al. [327] reported that °Brix of organic strawberries was 61.6% higher than conventional strawberries. Similarly , Oliveira et al. [328] showed that soluble solid content of conventional tomato fruits was 56% lower than organic fruits. Besides, Kobi et al. [329] reported that phenolic compounds, anthocyanin concentration and total soluble solids were higher in organically-grown strawberry plants than conventional one. In another study, Krolow et al. [330] observed higher °Brix and anthocyanins in organic compared with conventional strawberries. Increasing gustatory and

health components of fruits under organic farming is often related to stress conditions that result in an increase of secondary metabolites [9, 94, 325, 326].

Difference observed between our work and published studies may be explained by the fact that plants grown in the greenhouse had all the same environmental growing conditions.

CONCLUSION

Many different responses of biostimulants between organic and conventional growing management as well as between greenhouse and high tunnel growing systems were observed during this study. These differences may be related to the initial soil biological properties (e.g. organic vs conventional) and the presence of abiotic stresses that may have occurred (e.g. temperature, light, soil water content).

During the greenhouse experiment, we have determined the effects of different biostimulants to find out the most promising ones that were then studied under high tunnels. We have also studied the impact of different biostimulants under organic and conventional crop management. Our greenhouse experiment has shown that most biostimulants increased soil CO_2 efflux, while their impact on soil activity, expressed by the FDA, was only observed for the high tunnel plants treated with the mixture of mycorrhiza and bacteria. In both experiments, biostimulants did not improve the plant nutrient uptake as no significant increase was observed between the leaf macronutrient content of treated and control plants. Similarly, no beneficial effect of biostimulants was observed for the photosynthetic performance parameters (Fv/Fm, PI, SPAD, A_{max}, Rd and Φ). In contrast some negative impacts may occur as observed for the SPAD value for organic plants treated with bacteria and the Φ of high tunnel plants treated with citric acid.

Regarding the plant agronomic performance, our greenhouse and high tunnel experiments have shown that citric acid or citric and lactic acids are promising biostimulants in terms of plant growth and productivity compared with untreated organically- or conventionally cultivated plants. In fact, citric acid did increase the marketable yield of plants grown in the greenhouse, while all biostimulants have increased the number of flowering stalks (although mixture of mycorrhiza and bacteria were not significant at P=0.05) and the shoot fresh and dry biomass of high tunnel plants. On the other hand, we have observed that the mixture of mycorrhiza and bacteria may have a negative impact on crop productivity of conventionally grown plants (e.g. high tunnel experiment), while the opposite was observed for the organically-grown plants. Consequently, the clear benefits of using the studied biostimulants on plant growth and crop productivity, for both conventional and organic crop management, were mitigated, and depend on the environmental growing conditions. According to these results, we can conclude that our first hypothesis that "*In organic and conventional growing systems, biostimulants (seaweed extract, Trichoderma spp., mycorrhiza, nitrogen-fixing endosymbiosis bacteria, endosymbiotic nitrogen scavengers, phosphates and potassium*

solubilizing bacteria as well as organic acids) increase plant development and crop productivity by improving plant nutrient uptake" was not validated, although some benefits on plant growth and productivity were observed.

In terms of fruit quality, our results clearly demonstrated that biostimulants may improve the health value of the berries when expressed as anthocyanins or total polyphenols. In fact, for plants grown under higher tunnels, all biostimulants increased the fruit anthocyanin content by 34 to 56%. For plants grown under greenhouse, the mixture of mycorrhiza and bacteria treatment increased the anthocyanin (+25%) and total polyphenol (+40%) content of organically-grown plants, while citric acid increased the anthocyanin content (+24%) of conventionally grown plants. However, the studied biostimulants did not have a concluding impact on fruit total soluble sugar (Brix). *Trichoderma* under conventional greenhouse management increased the fruit total soluble sugars but only during two sampling periods (April 15th and May 1st), while the mixture of mycorrhiza and bacteria of organically-grown plants increased Brix during that time. These results confirm our second hypothesis that biostimulants may improve the berry quality under conventional and organic growing management, although a general statement cannot be made. Different results between the greenhouse and high tunnel experiments, however, may be explained by diverse environmental growing conditions such as light, temperature, and humidity. In addition, high-tunnels can be most stressful conditions than greenhouse by higher and/or large variations of temperature and higher pest pressure, which are well known to impact secondary components.

These results may have a significant impact on the berry industry by proposing a sustainable approach to improve plant growth, crop productivity and fruit quality. However, different results observed between the performance of biostimulants for plants grown under greenhouses and high tunnels indicate the needs for further studies, which should be conducted over several growing seasons, and under different abiotic conditions. It is also essential to optimize the application dose, frequency and method (foliar, drench or soil applications). The cost and profitability of using biostimulants for both types of growing systems should also be considered.

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ANNEXE A

Annex A. 1 Production inputs (agricultural chemicals), disease and insect control practices during the growing period in high-tunnel- summer 2018.

Date of application	Product name	Dose (in hectare)	Application method
19 May 2018	PRISTINE	1.6kg	Air
2 June 2018	OBERON	1L	Air
5 June 2018	MAESTRO	2.75kg	Air
11 June 2018	LUNA TRANQUILITY	1.2L	Air
18 June 2018	NEALTA	1L	Air
21 June 2018	FLINT	140g	Air
21 June 2018	BELEAF	200g	Air
28 June 2018	OBERON	1L	Air
29 June 2018	SWITCH	975g/ha	Air
4 July 2018	SWITCH	975g/ha	Air
18 July 2018	PRISTINE	1,3 kg	Air
18 July 2018	BELEAF	160 g	Air
27 July 2018	SERCADIS	500 ml /ha	Air
1 August 2018	SUCCESS	182 ml	Air
1 August 2018	LUNA TRANQUILITY	1,2 L	Air
7 August 2018	SWITCH	975g/ha	Air
7 August 2018	DELEGATE	280 g	Air

ANNEXE B

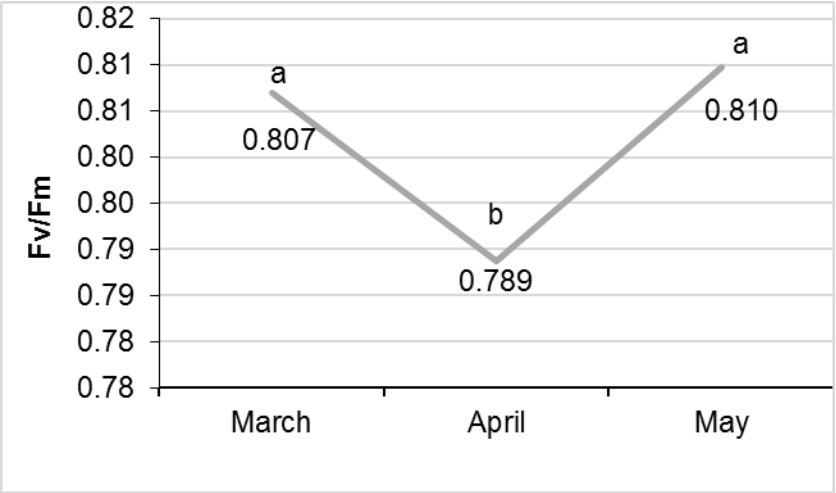
Annex B.1 Microbial activity and CO_2 efflux of strawberries cultivated in greenhouse under different combination of growing systems and biostimulants.

Treatments		**FDA (ug/g/h)**	**CO_2 efflux ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**
Conventional	**CONTROL**[z]	530.25 cd	4.29 g
	SEAWEED	507.25 cd	6.43 f
	TRICHO	381.73 d	9.81 de
	MYC	626.99 bc	10.91 cd
	BACT	514.51 cd	7.24 f
	MYC+BACT	454.28 cd	6.55 f
	CITRIC	436.43cd	7.48 ef
	CONTROL	884.95 a	11.42 cd
	SEAWEED	881.77 a	16.42 b
Organic	MYC	906.83 a	27.07 a
	BACT	758.57 ab	16.67 b
	MYC+BACT	898.55 a	17.23 b
	MYC+BACT/LF	555.85 cd	11.94 cd
	CITRIC	830.04 a	15.46 bc
Growing systems	Conventional	493 b	7.53 b
	Organic	817 a	16.60 a
P values			
Biostimulant (B)		<0.001	<0.001
Conventional vs Organic		<0.001	<0.001

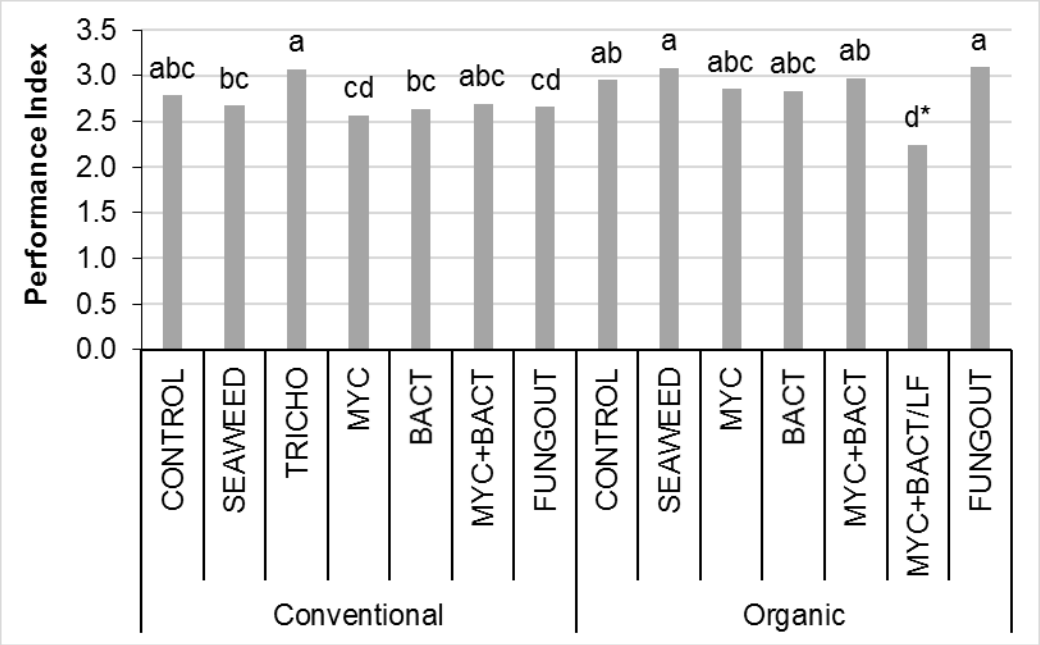
[z]CONTROL= without biostimulant; SEAWEED= seaweed extract; TRICHO = *Trichoderma*, MYC= mycorrhiza *Rhizoglomus irregulare*; BACT= three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT= combination of treatments MYC and BACT; MYC+BACT/LF= combination of treatments MYC and BACT with low fertilization; CITRIC= Citric acid (citric acid-based formulation., AEF GLOBAL Inc.).

[x]means of the same column with different letters are significantly different at $P<0.05$.

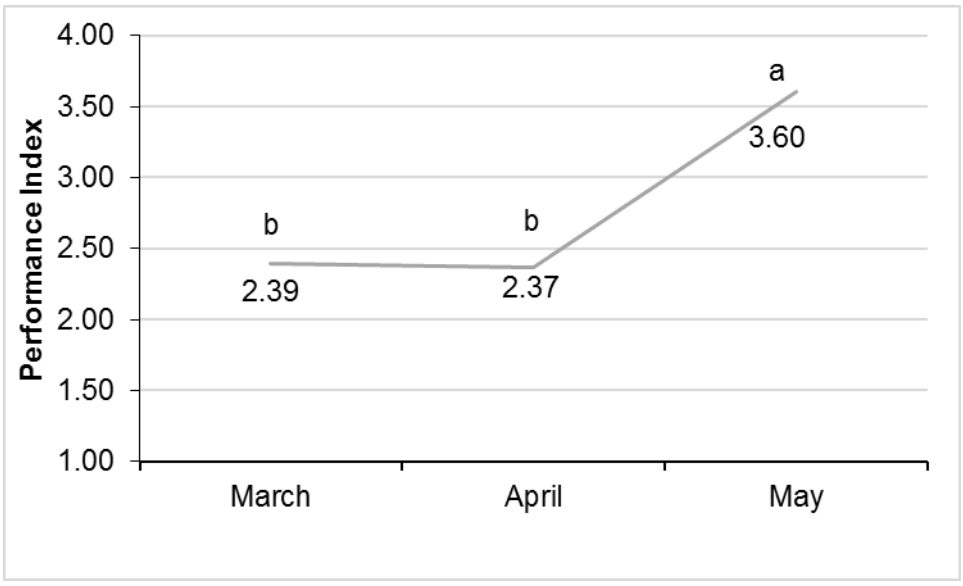
*Treatments are different from their respective control.



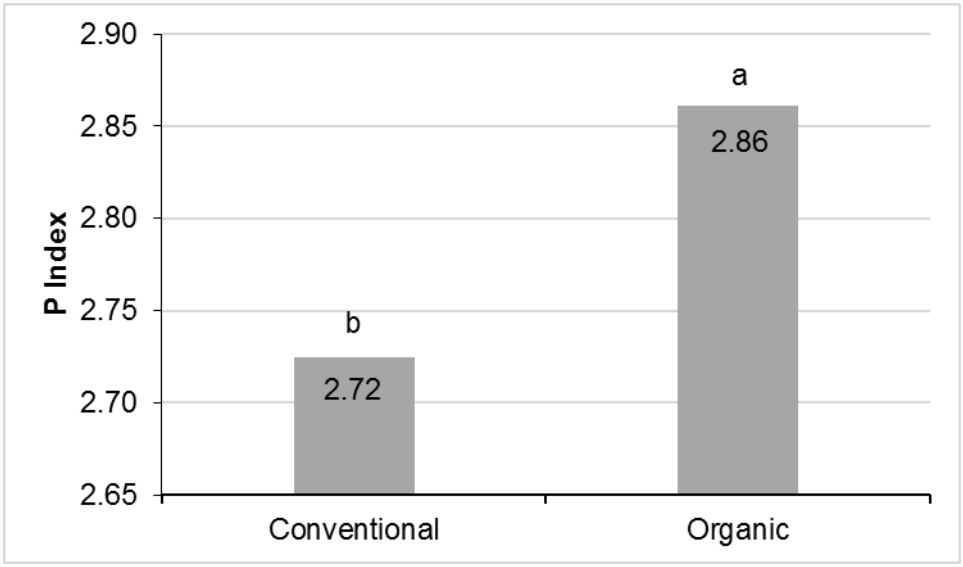
Annex B.2 The maximum quantum efficiency of photosystem II (Fv/Fm) variation on strawberry leaves of plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05).



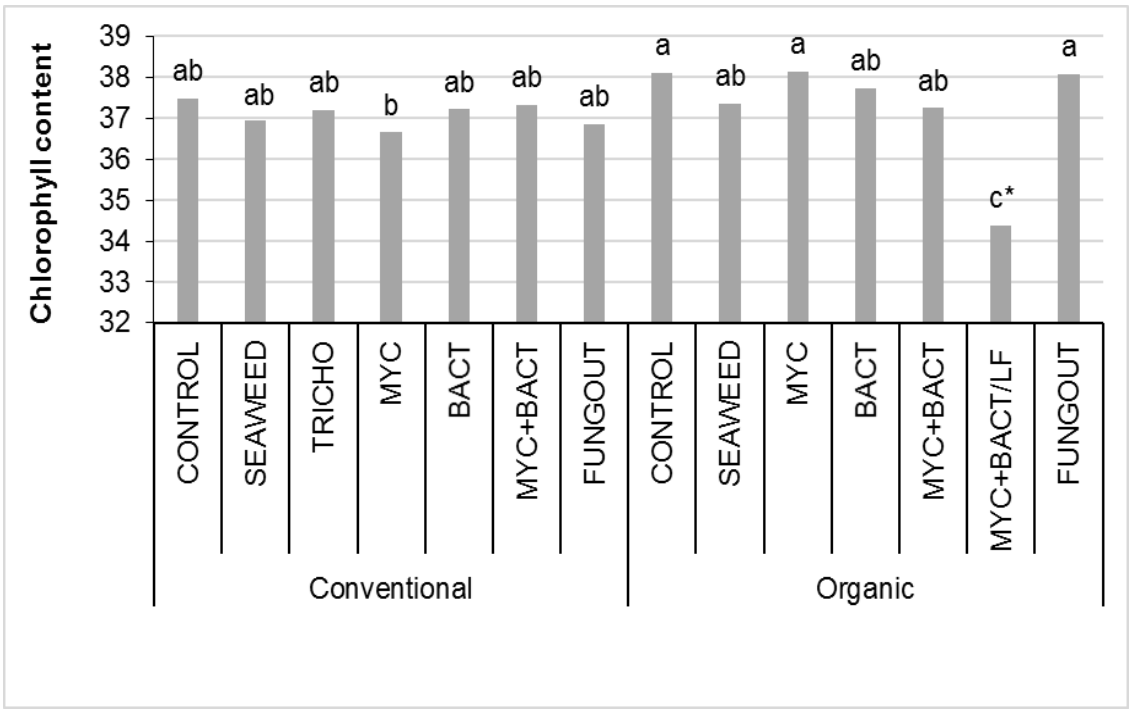
Annex B.3 The performance index variation on strawberry leaves of plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=45).



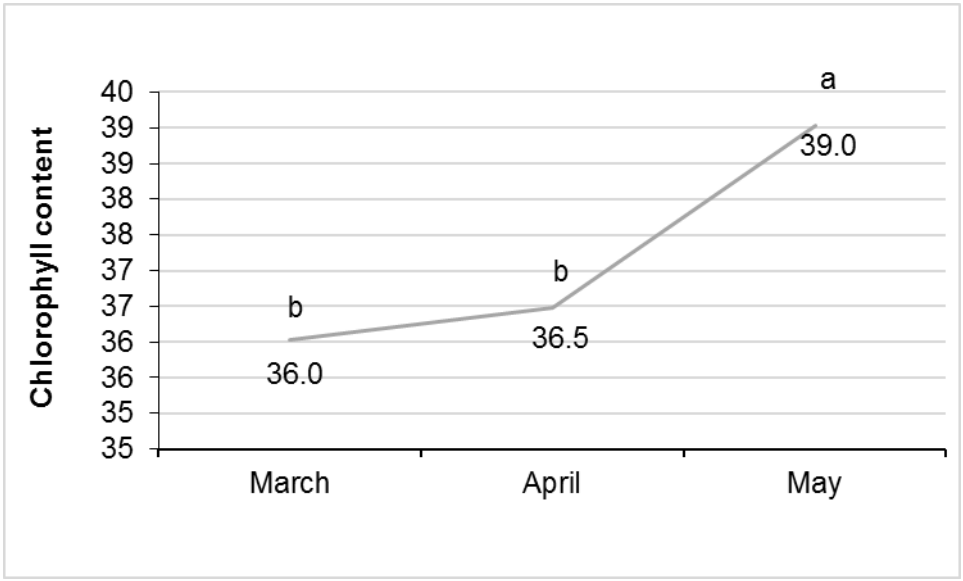
Annex B.4 The performance index (P Index) variation on strawberry leaves of plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05).



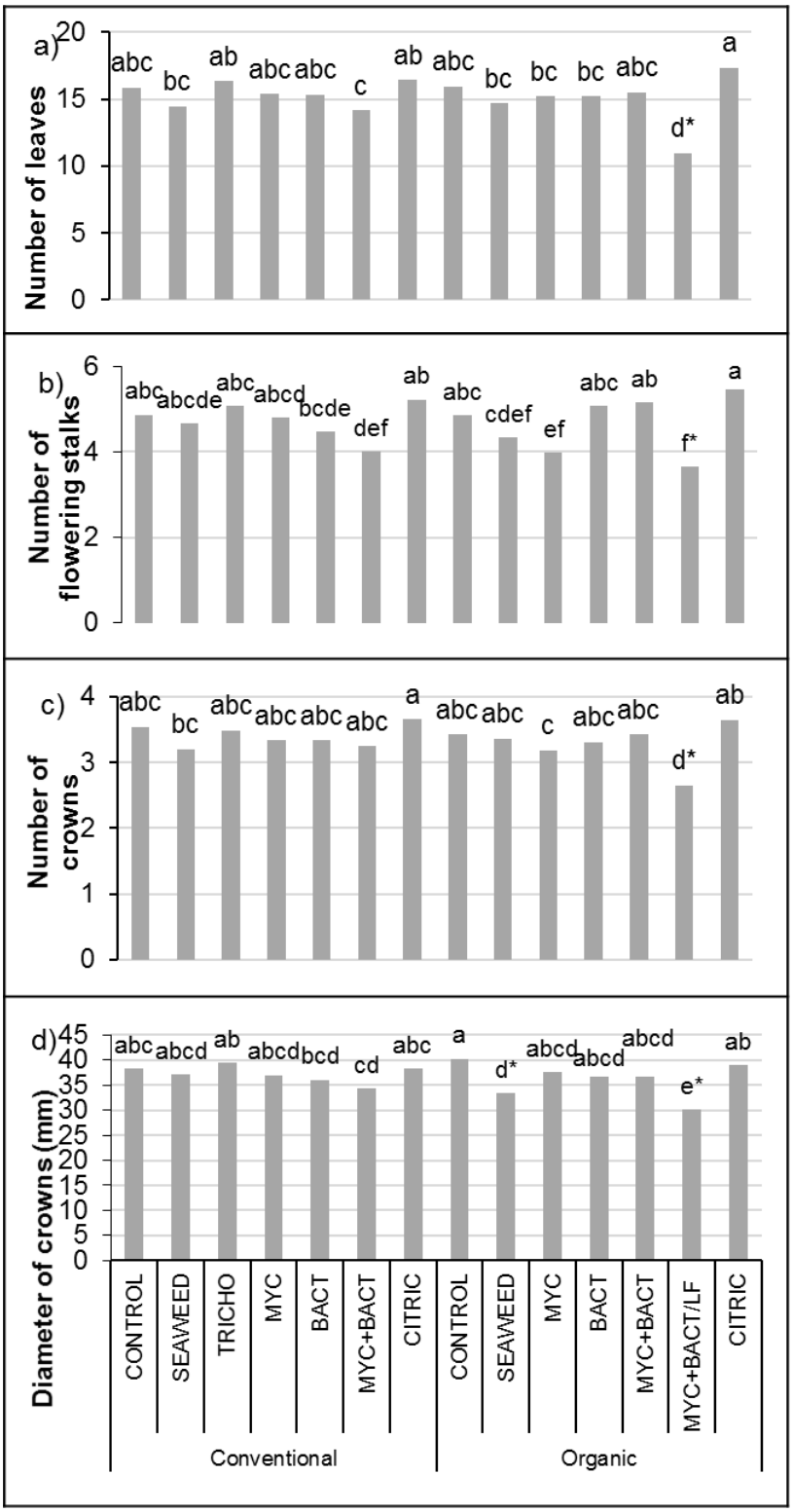
Annex B.5 The performance index (P Index) variation of strawberry plants by growing system during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05).



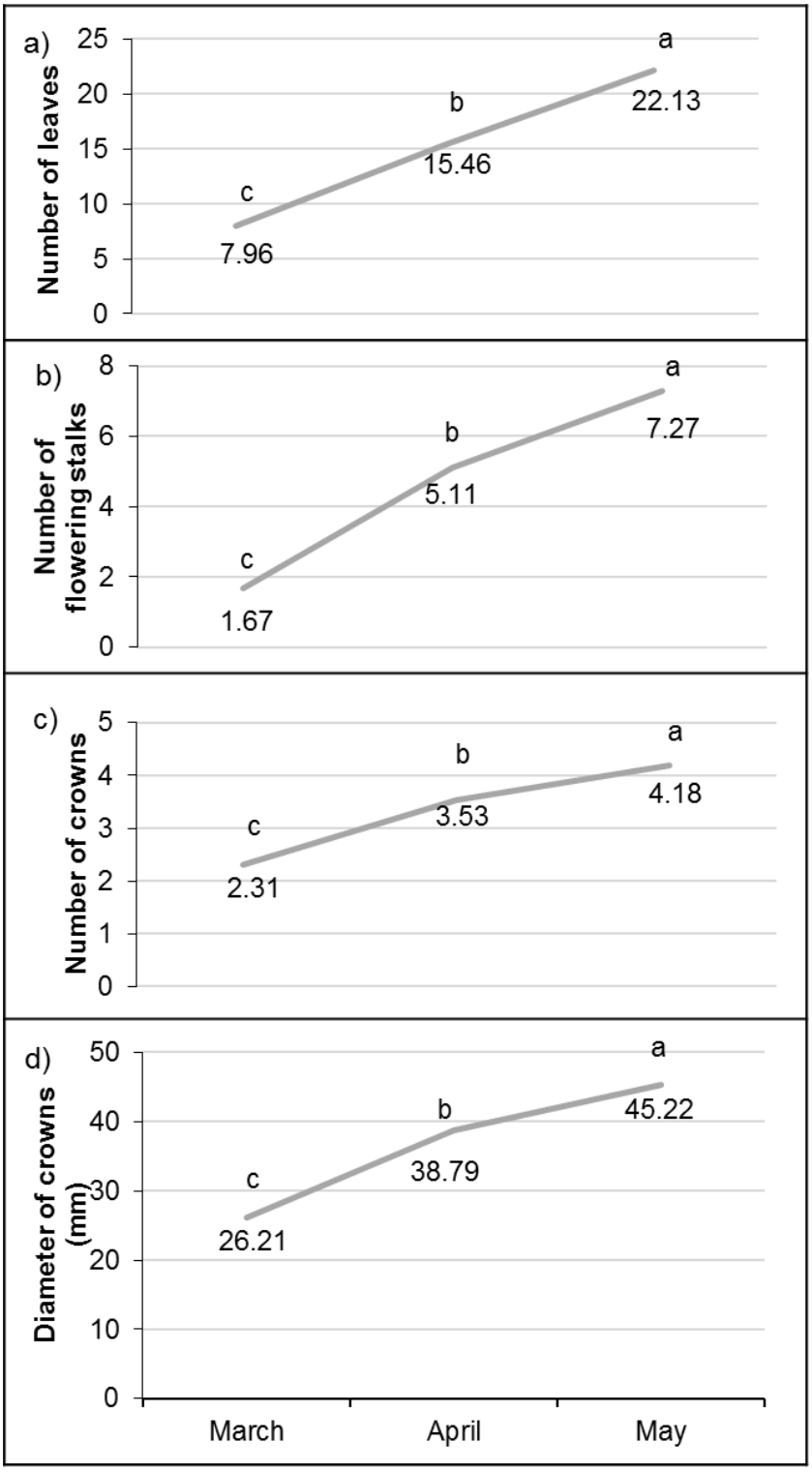
Annex B.6 Chlorophyll content variation on strawberry leaves of plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=45).



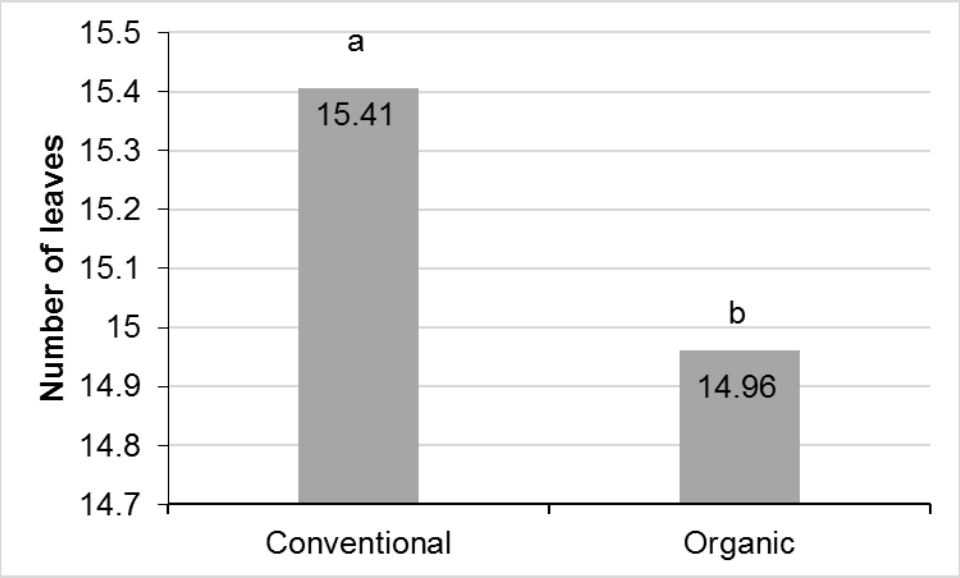
Annex B.7 Chlorophyll content variation on strawberry leaves of plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05).



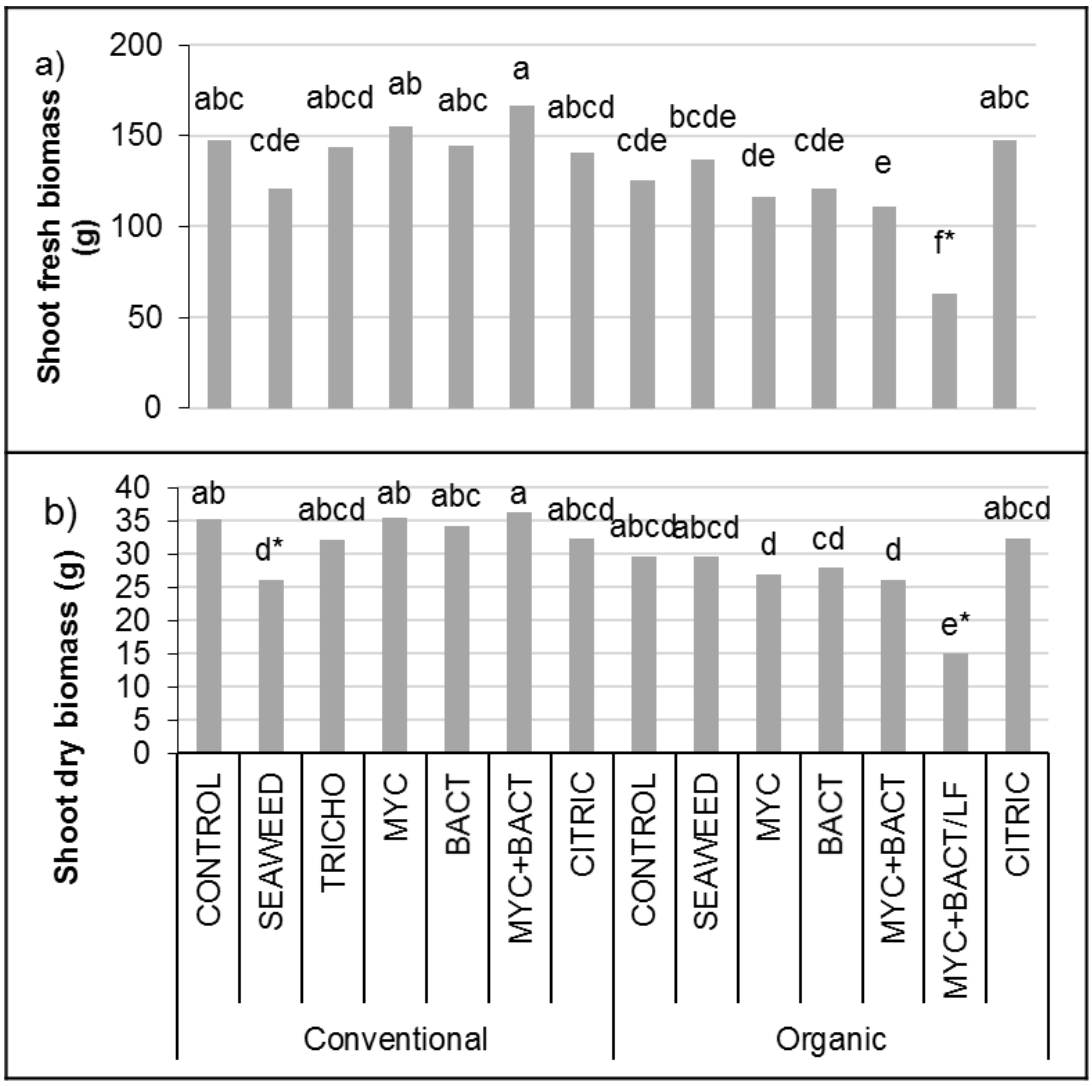
Annex B.8 Growth parameters variation of strawberry plants treated with studied biostimulants a) number of leaves, b) number of flowering stalks, c) number of crowns, d) diameter of stalks. Means followed by the same letter are not significantly different (P≤ 0.05) (n=15).



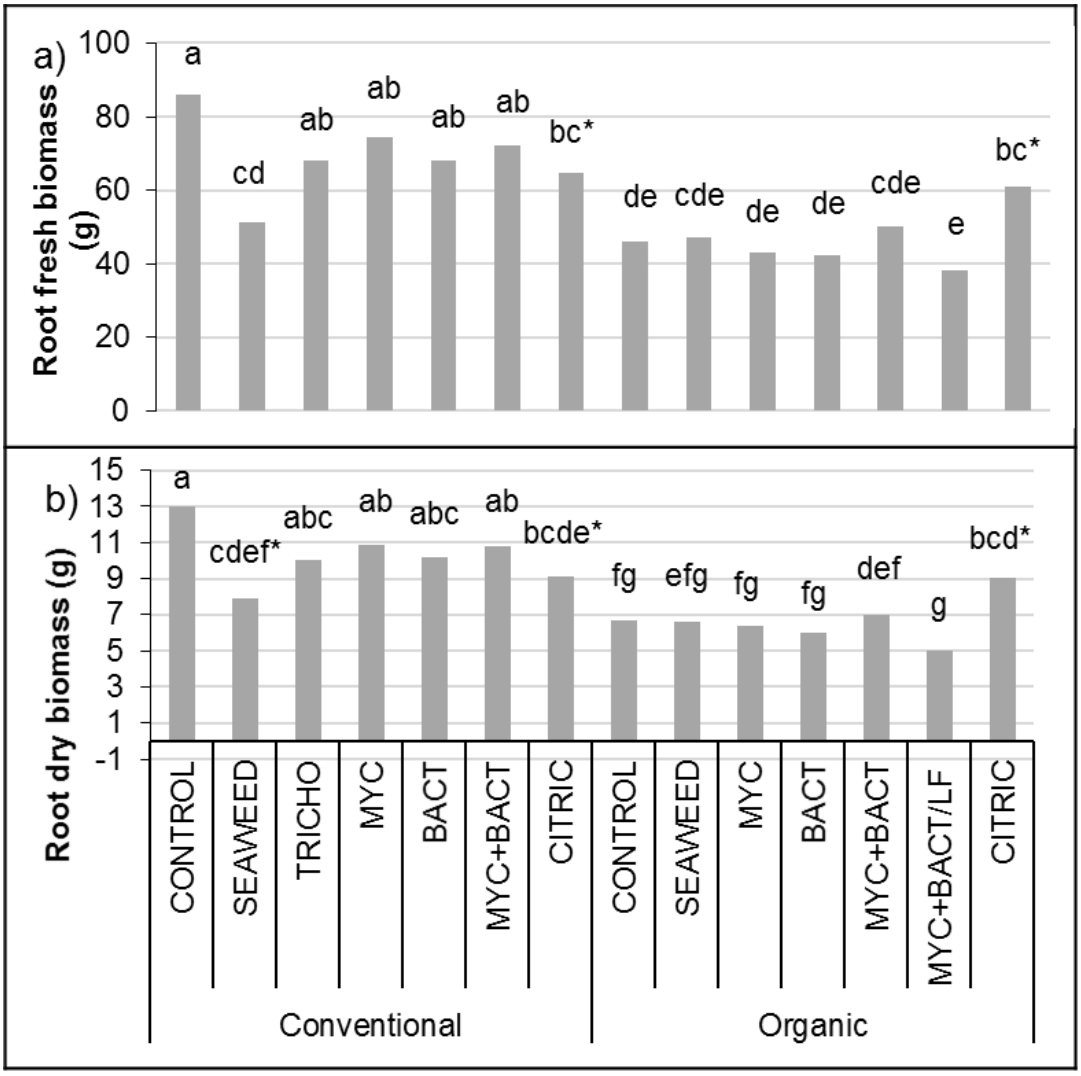
Annex B.9 Growth parameters variation of strawberry plants a) number of leaves, b) number of flowering stalks, c) number of crowns, d) diameter of stalks during the experimental period (winter 2018). Means followed by the same letter are not significantly different (P≤ 0.05) (n=45).



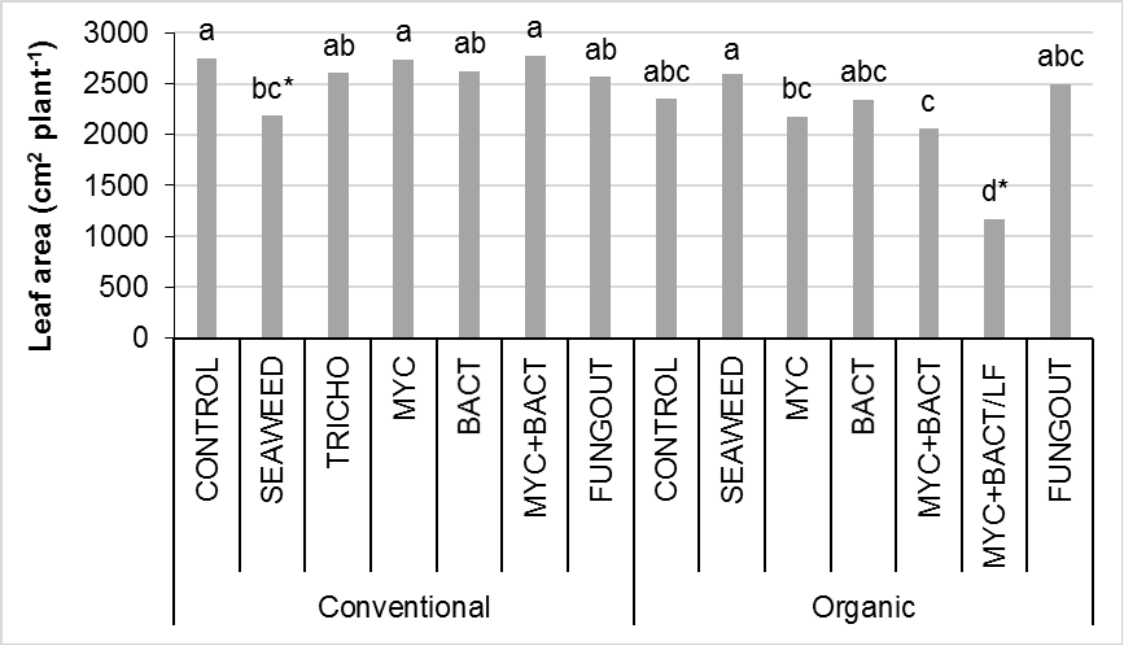
Annex B.10 Number of leaves variation of strawberry plants by growing system during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05).



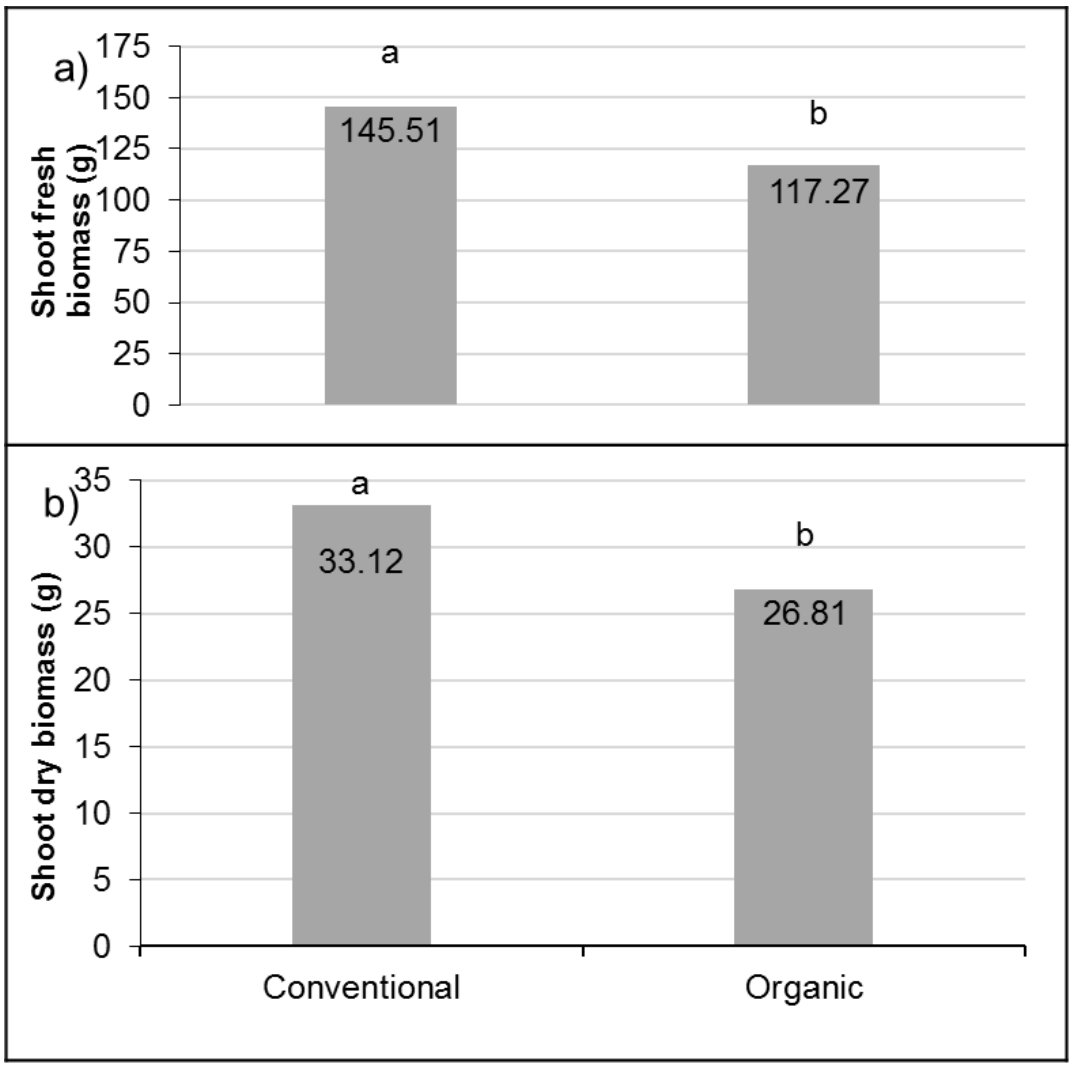
Annex B.11 Biomass variation of strawberry plants treated with studied biostimulants a) shoot fresh biomass and b) shoot dry biomass. Means followed by the same letter are not significantly different (P≤ 0.05) (n=45).



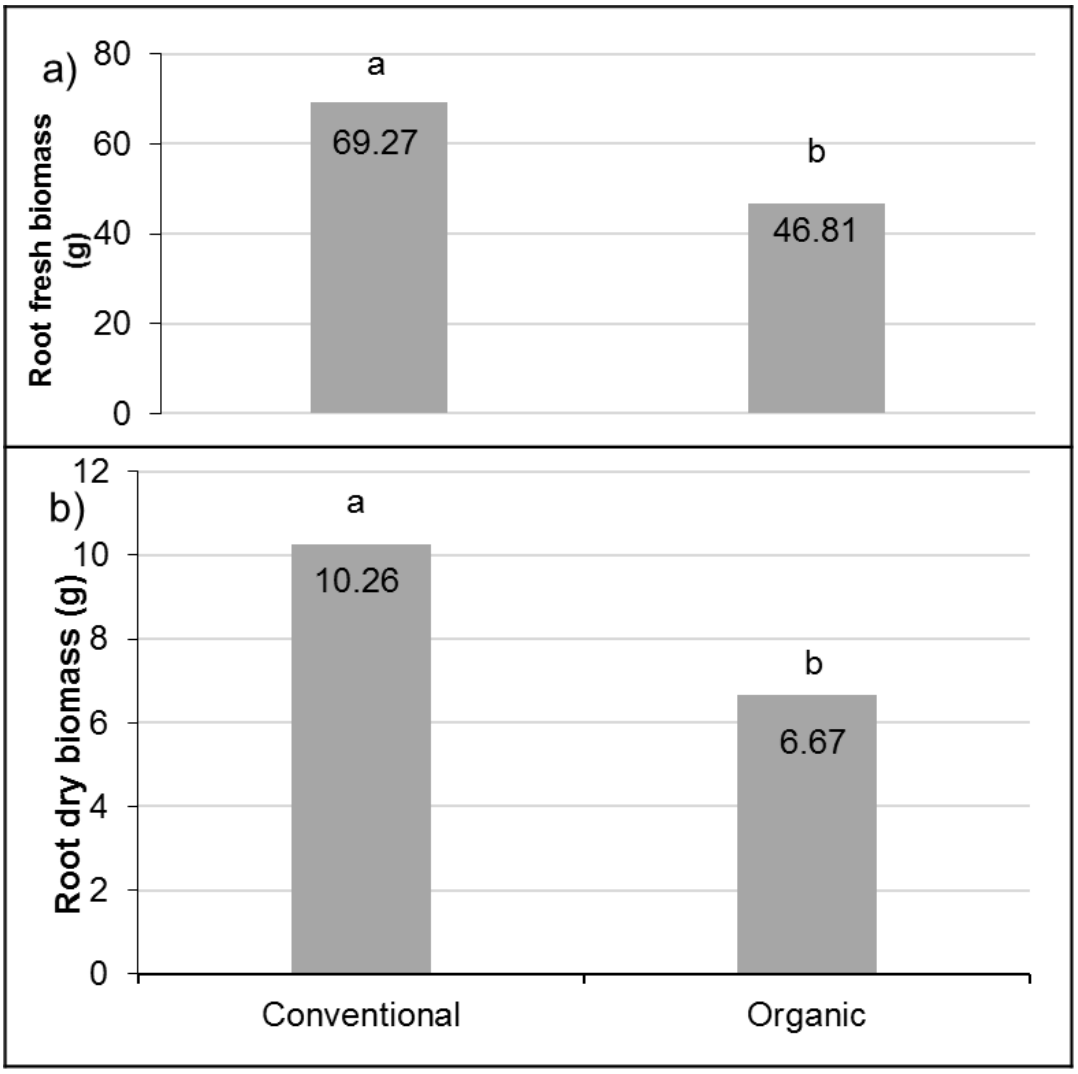
Annex B.12 Biomass variation of strawberry plants treated with studied biostimulants a) root fresh biomass and b) root dry biomass. Means followed by the same letter are not significantly different (P≤ 0.05) (n=45).



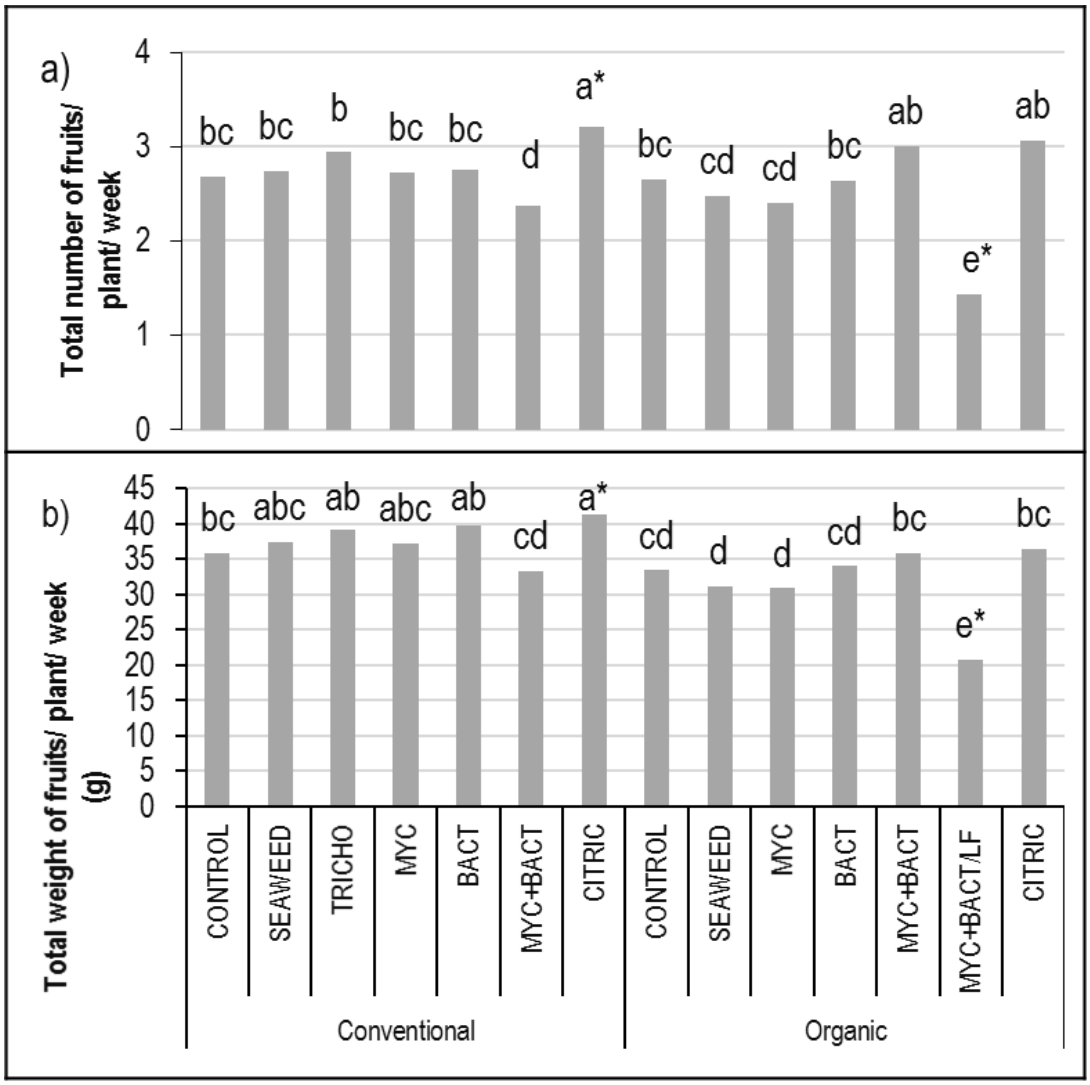
Annex B.13 Leaf area variation on strawberry leaves of plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=45).



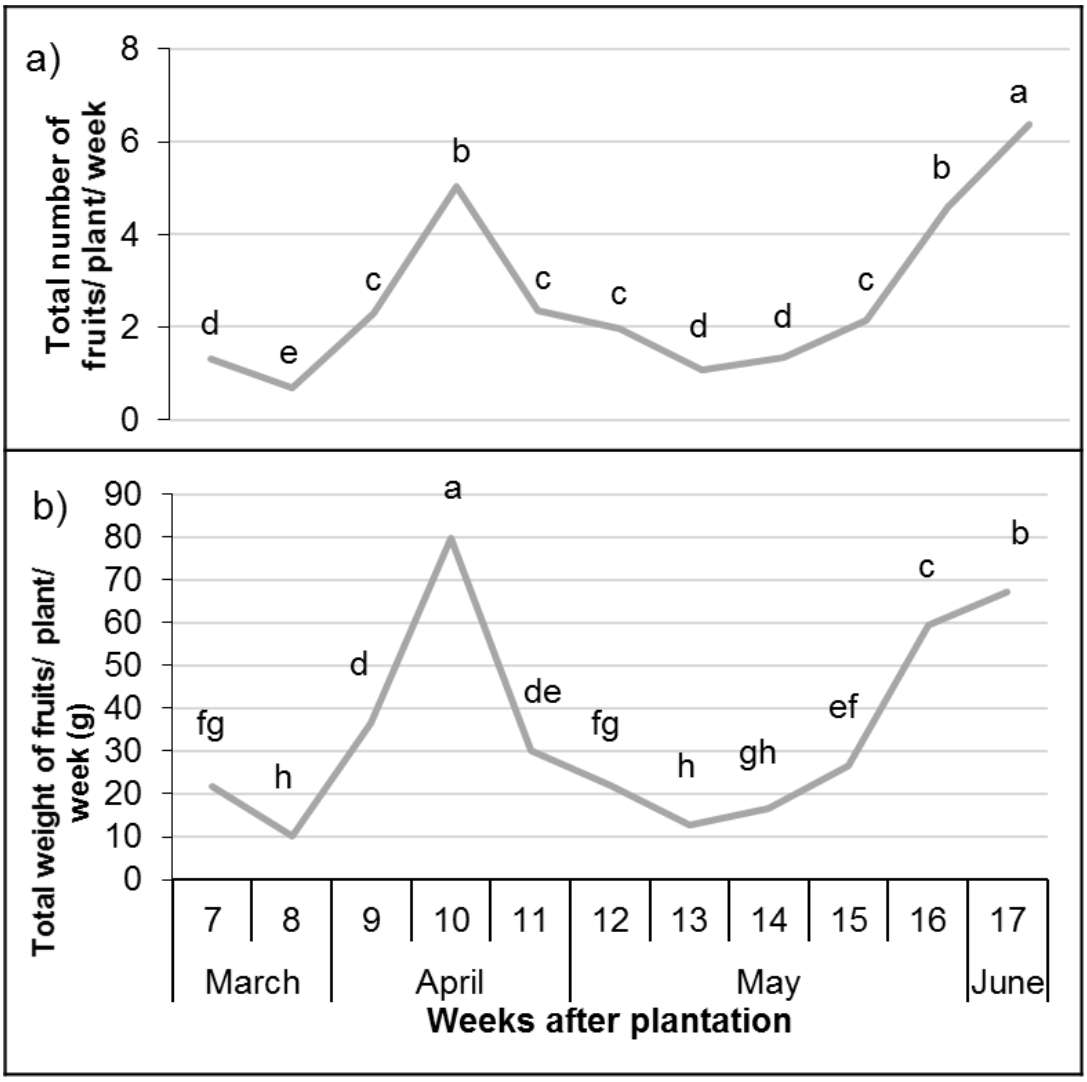
Annex B.14 Biomass variation of strawberry plants by growing system during winter 2018, a) shoot fresh biomass and b) shoot dry biomass. Means followed by the same letter are not significantly different (P≤ 0.05).



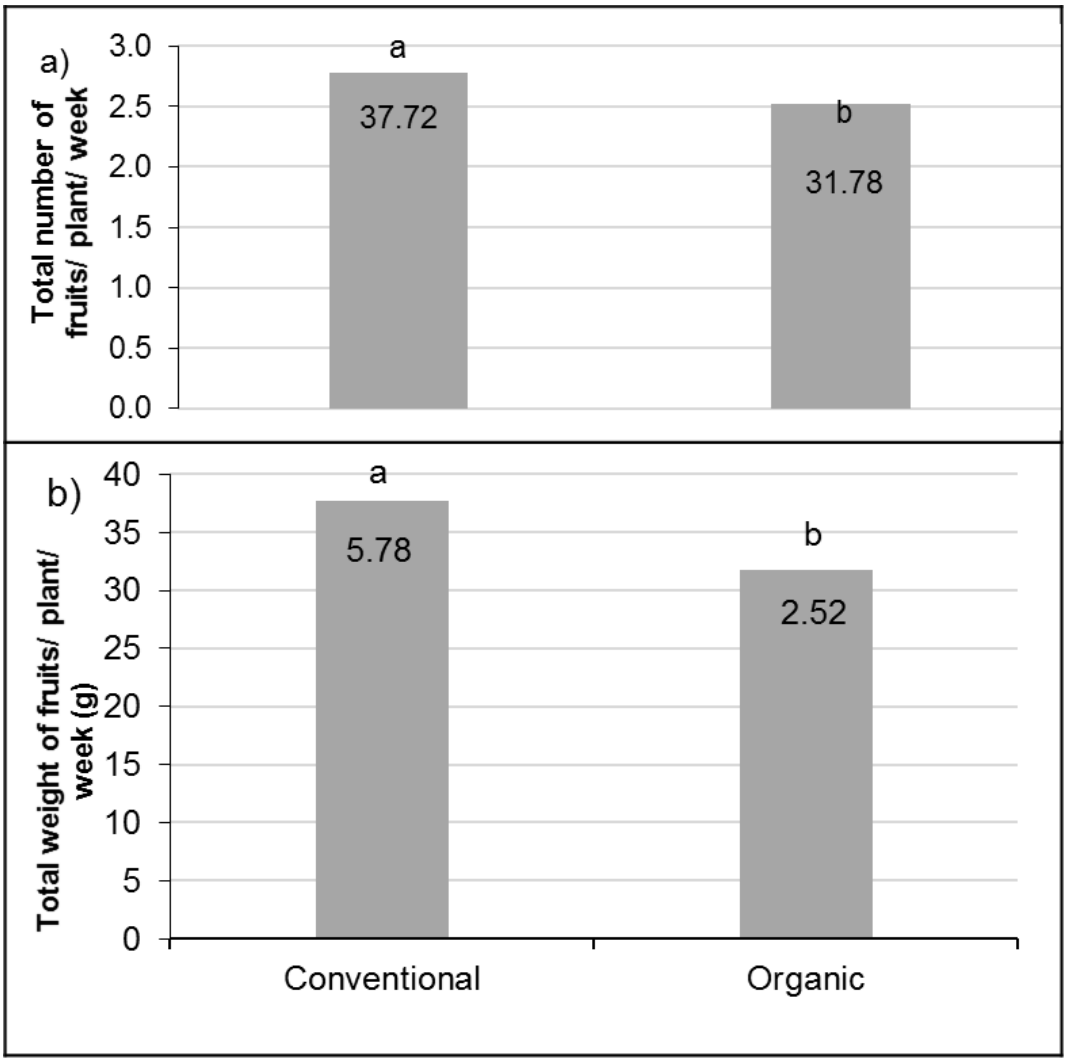
Annex B.15 Biomass variation of strawberry plants by growing system during winter 2018, a) root fresh biomass and b) root dry biomass. Means followed by the same letter are not significantly different (P≤ 0.05).



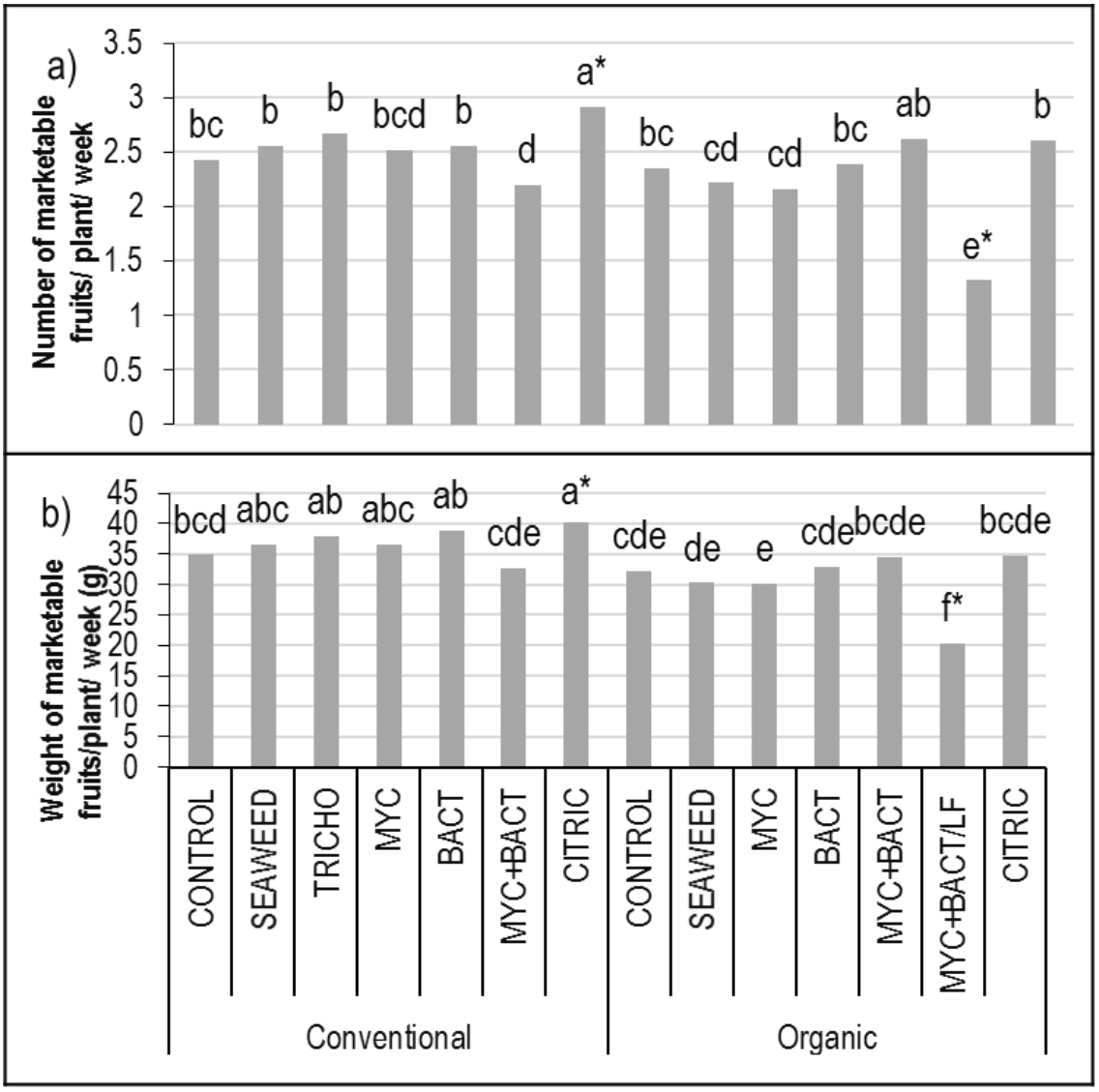
Annex B.16 Total yield (a) total number, (b) total weight variation on strawberry plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=55).



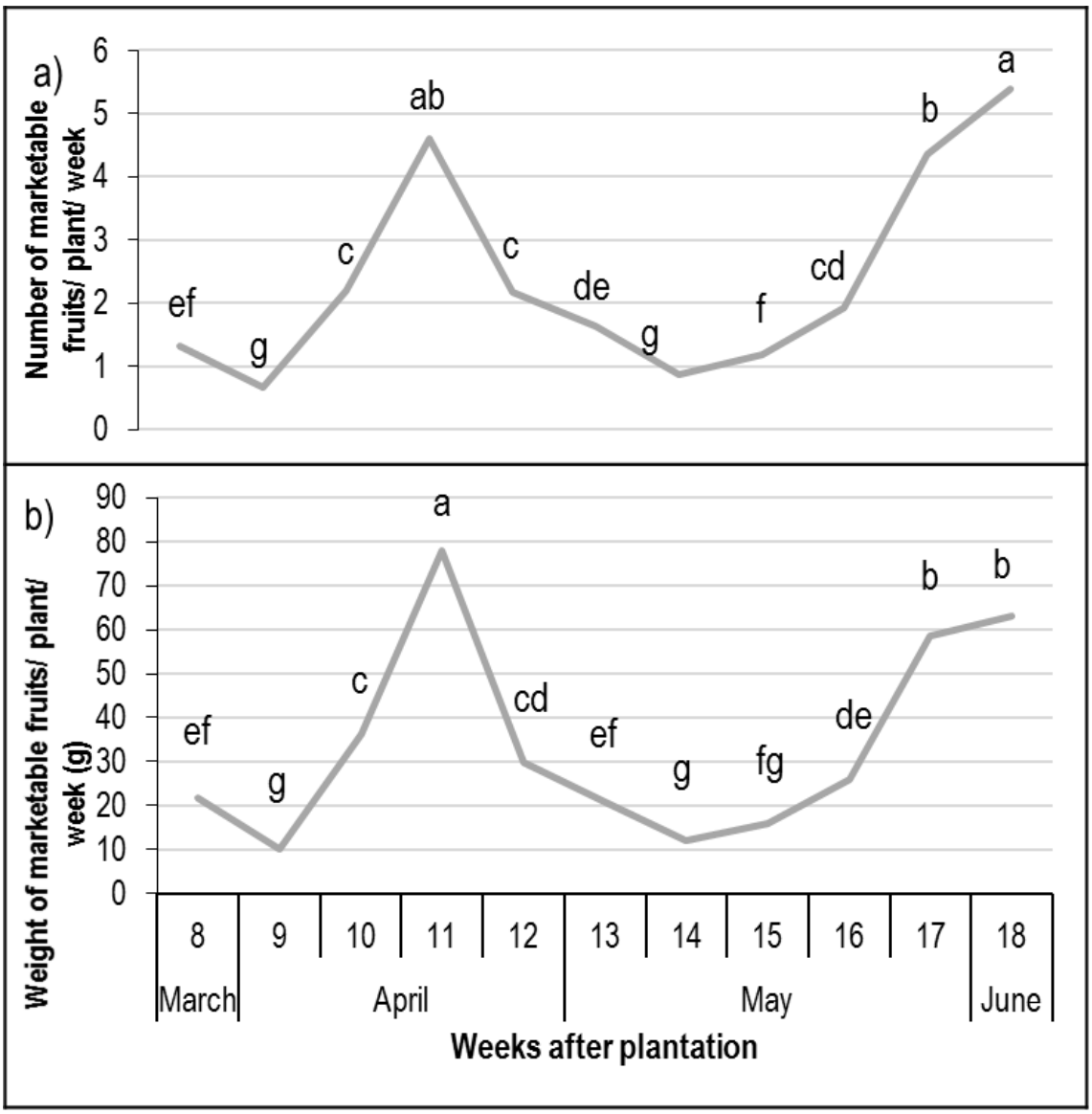
Annex B.17 Total yield (a) number, (b) weight variation of strawberry plants during the experimental period (winter 2018). Means followed by the same letter are not significantly different (P≤ 0.05).



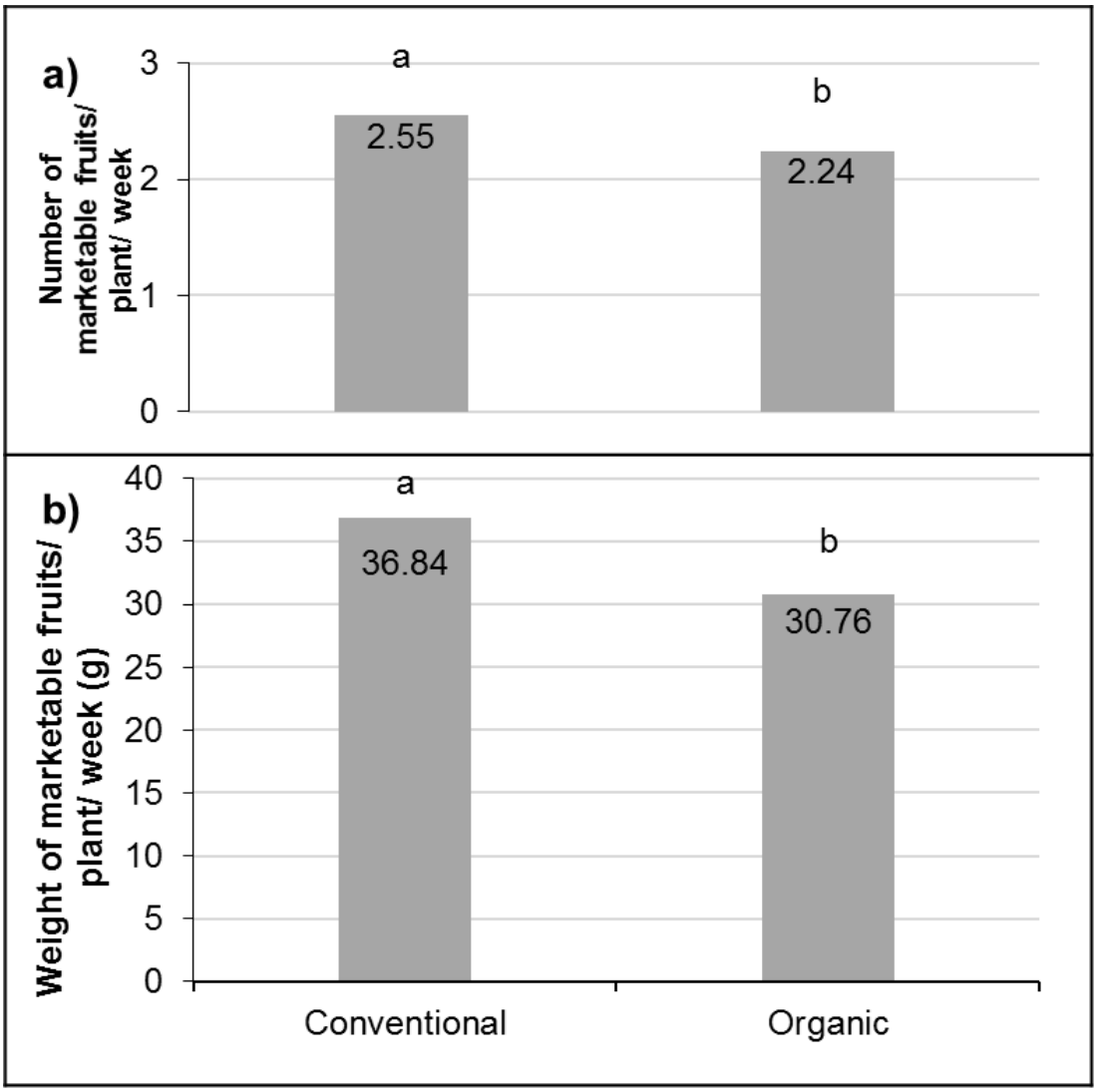
Annex B.18 Total yield (a) total number, (b) total weight of strawberry plants by growing system during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05).



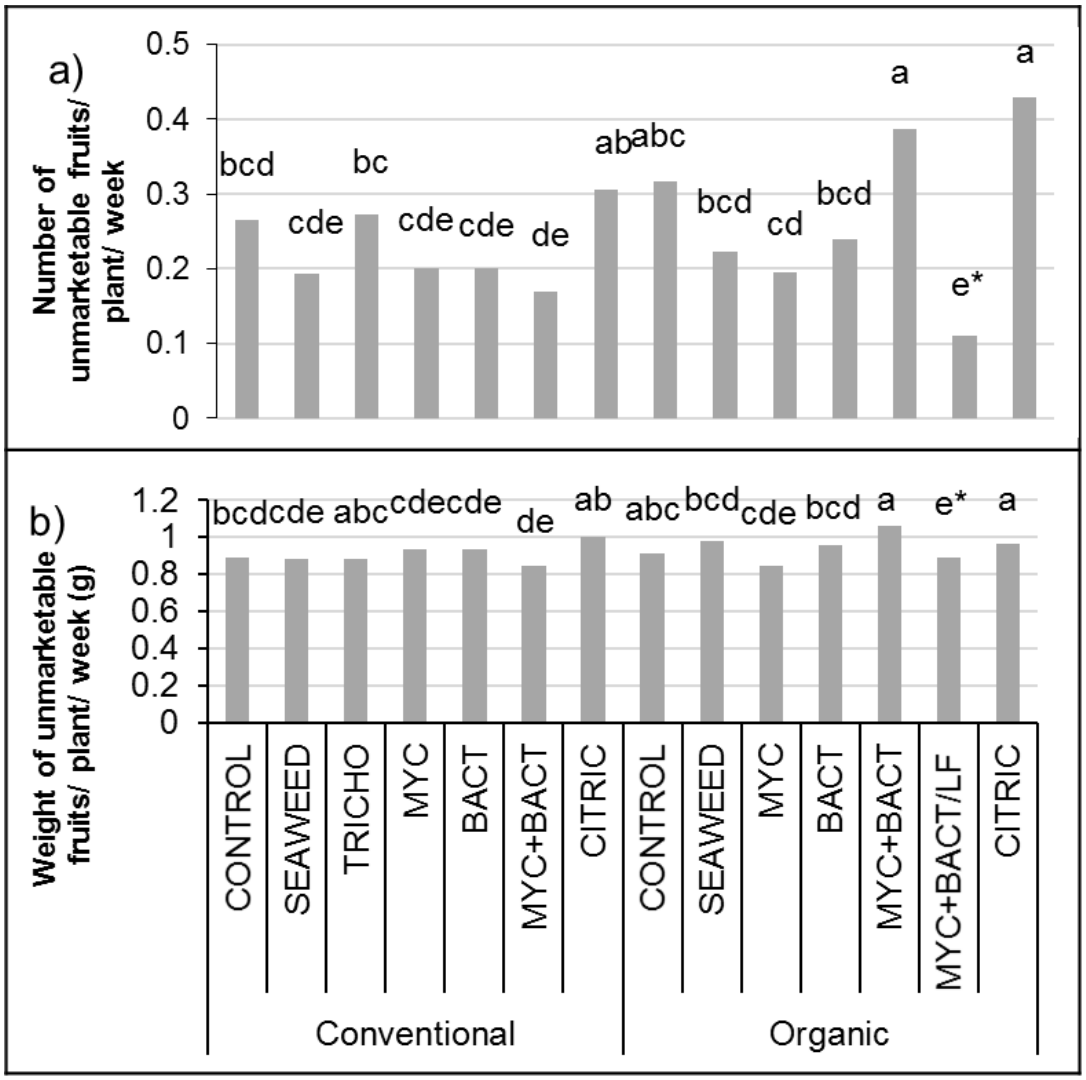
Annex B.19 Marketable yield (a) number, (b) weight variation on strawberry plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=55).



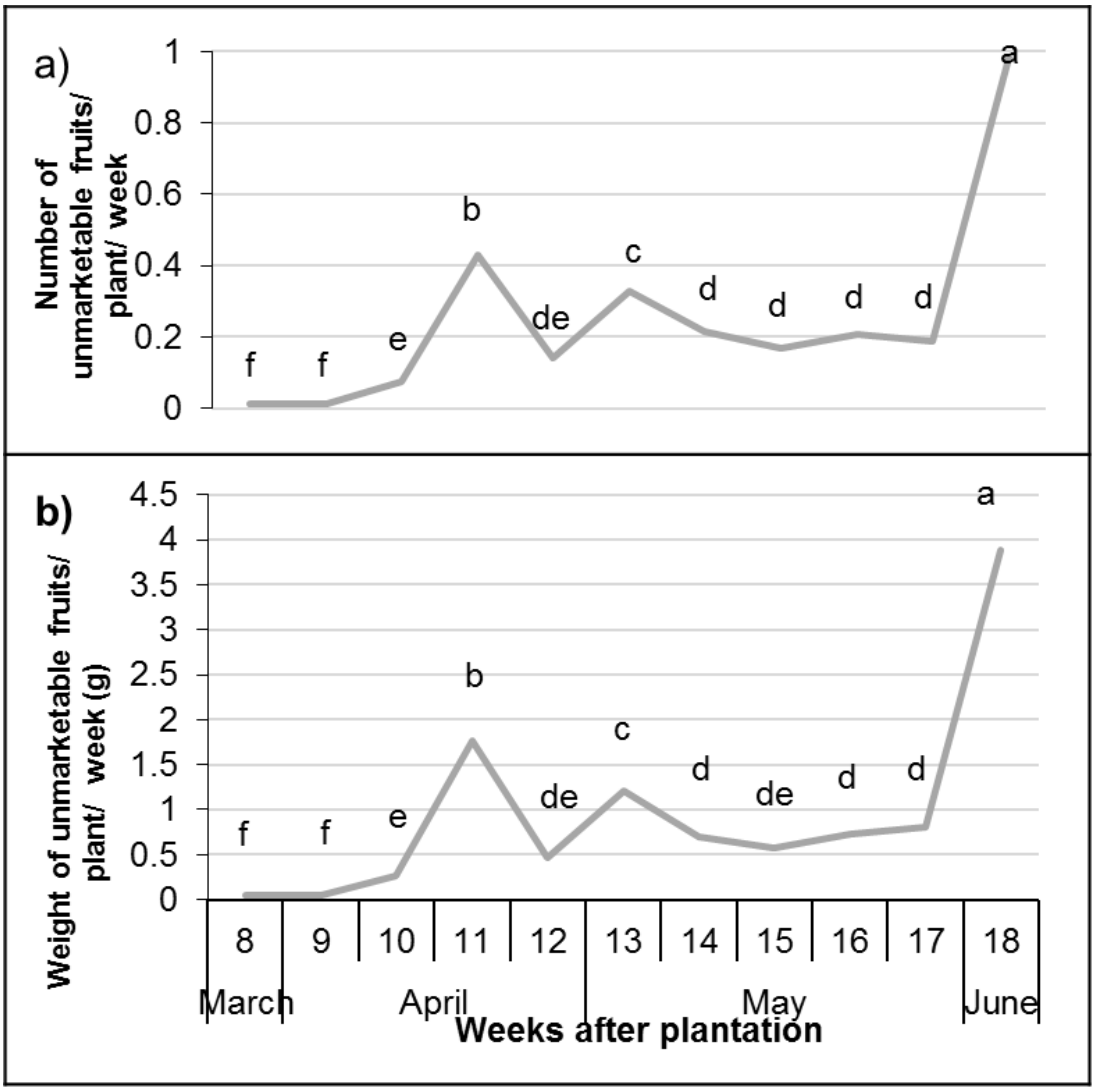
Annex B.20 Marketable yield (a) number, (b) weight variation of strawberry plants by growing system during winter 2018, a) root fresh biomass and b) root dry biomass. Means followed by the same letter are not significantly different (P≤ 0.05).



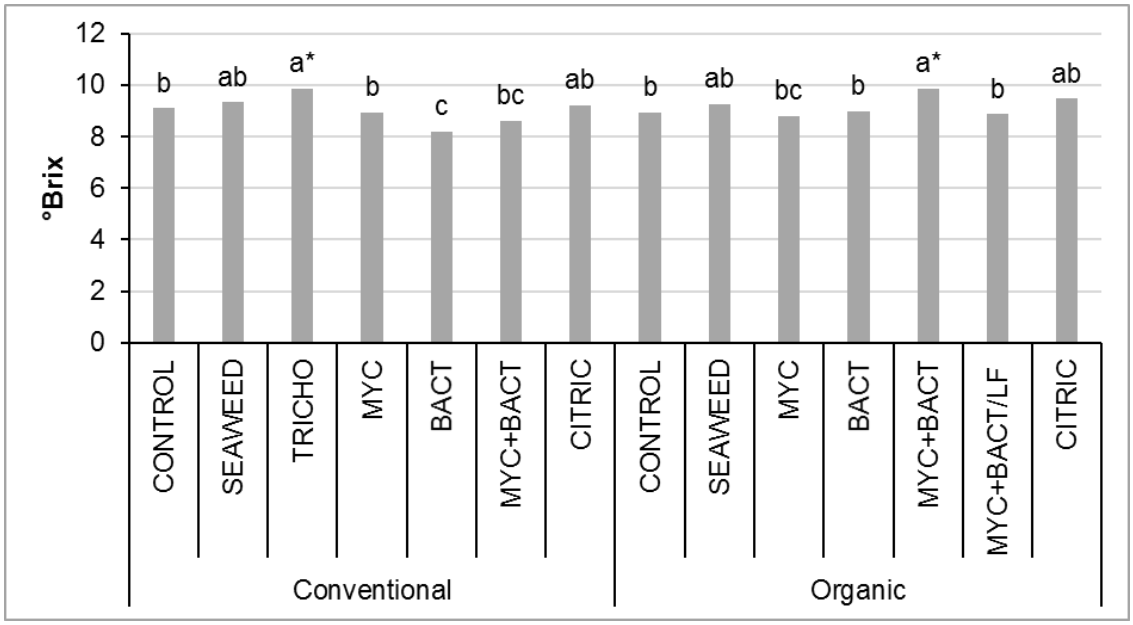
Annex B.21 Marketable yield (a) total number, (b) total weight of strawberry plants by growing system during winter 2018. Means followed by the same letter are not significantly different (P≤ 0.05).



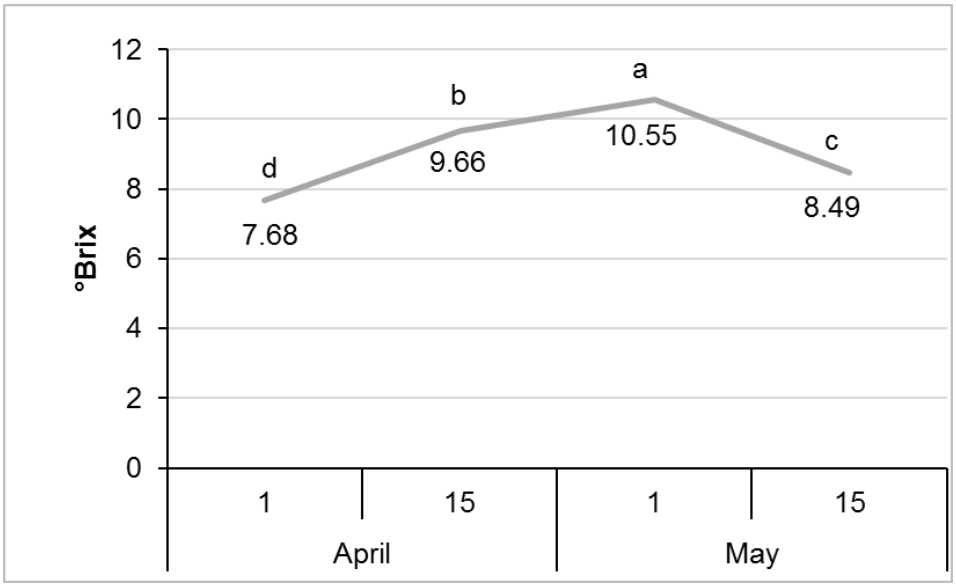
Annex B.22 Unmarketable yield (a) number, (b) weight variation on strawberry plants during the experimental period of winter 2018. Means with the same letter are not significantly different (P≤ 0.05) (n=55).



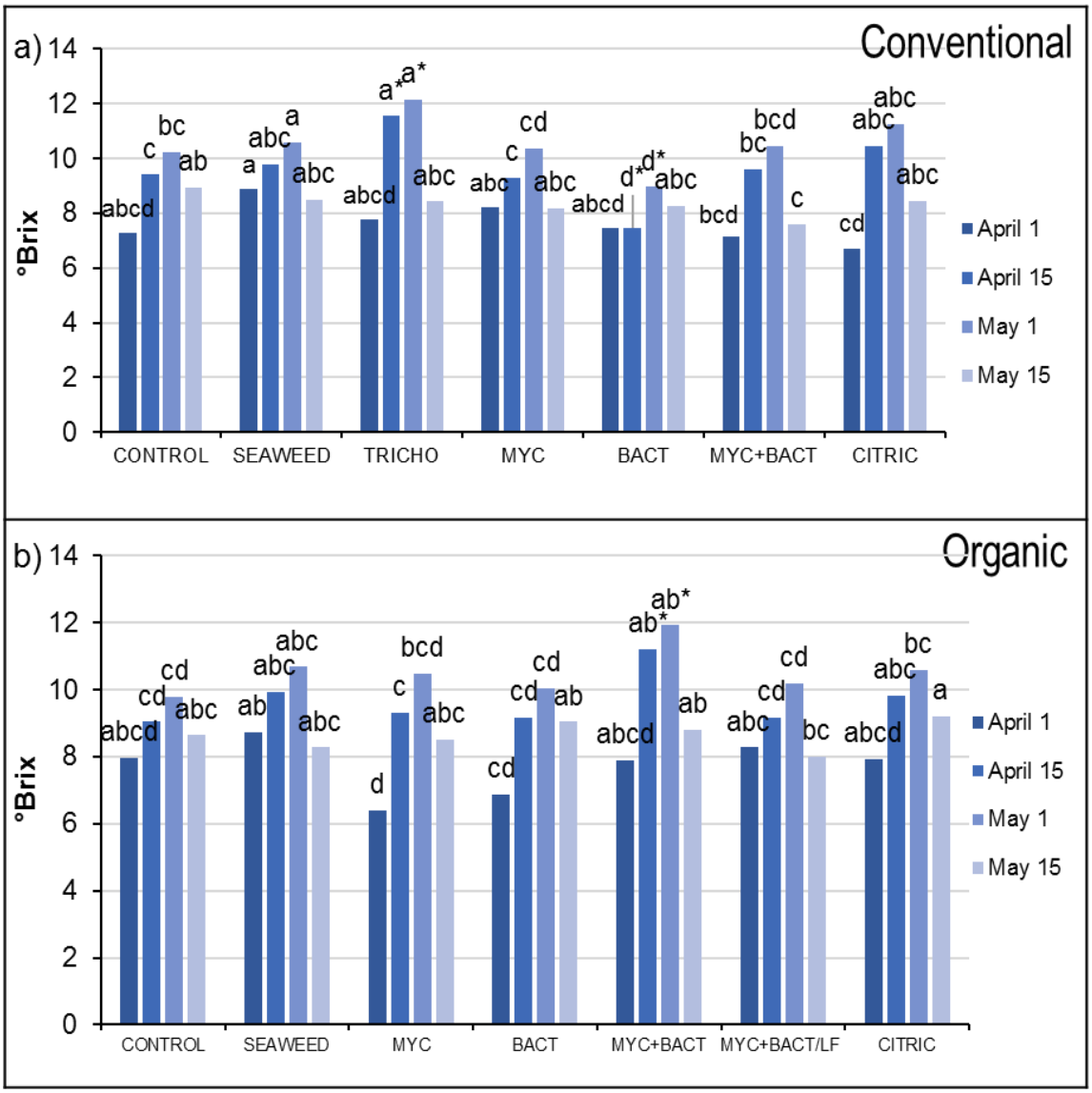
Annex B.23 Unmarketable yield (a) number, (b) weight variation of strawberry plants by growing system during winter 2018, a) root fresh biomass and b) root dry biomass. Means followed by the same letter are not significantly different (P≤ 0.05).



Annex B.24 The soluble sugar content (°Brix) of berries from strawberry plants treated with biostimulants and grown under conventional and organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05).

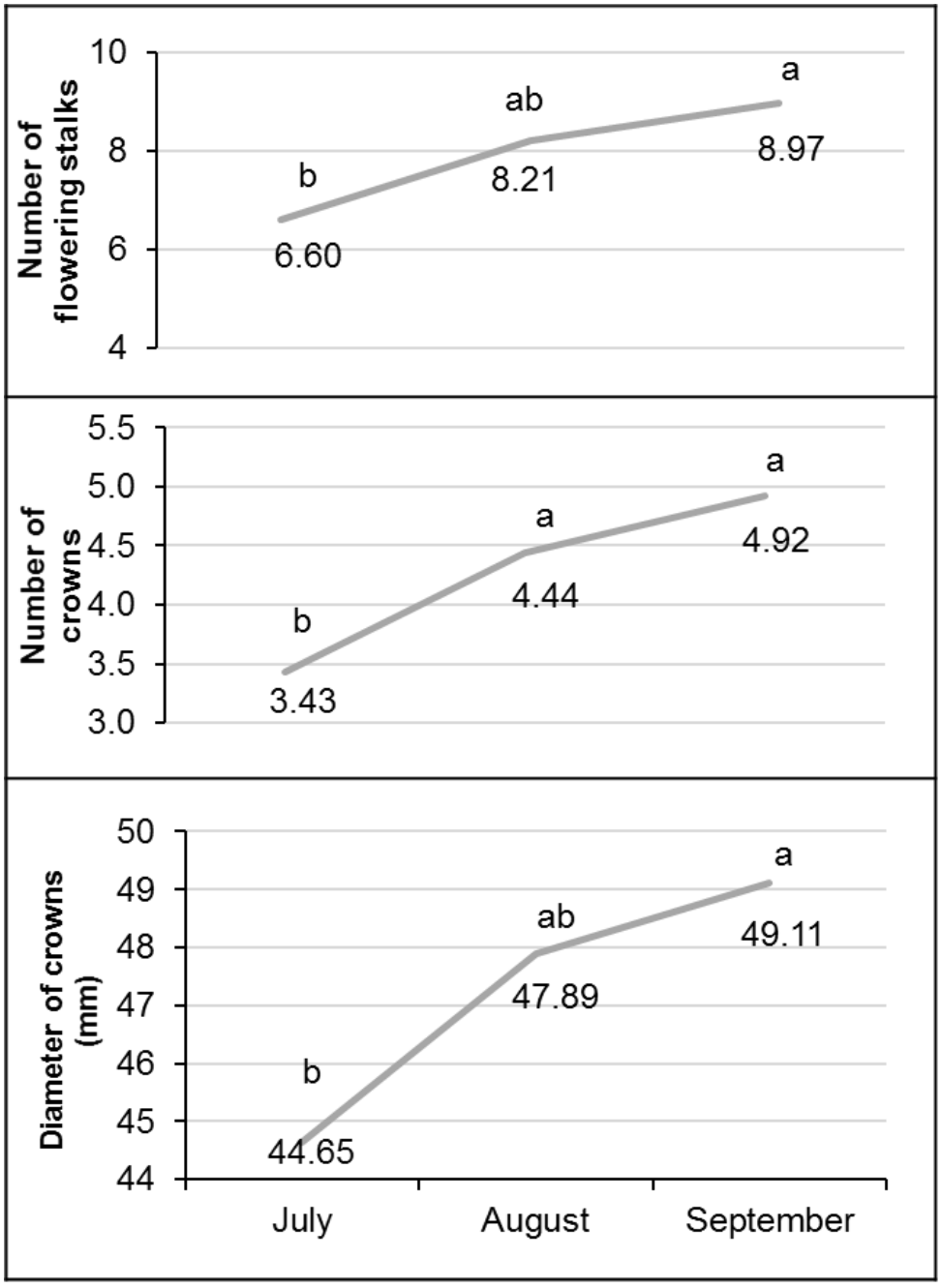


Annex B.25 Effect of time on the soluble sugar content (°Brix) of berries from strawberry plants treated with biostimulants and grown under conventional and organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05).

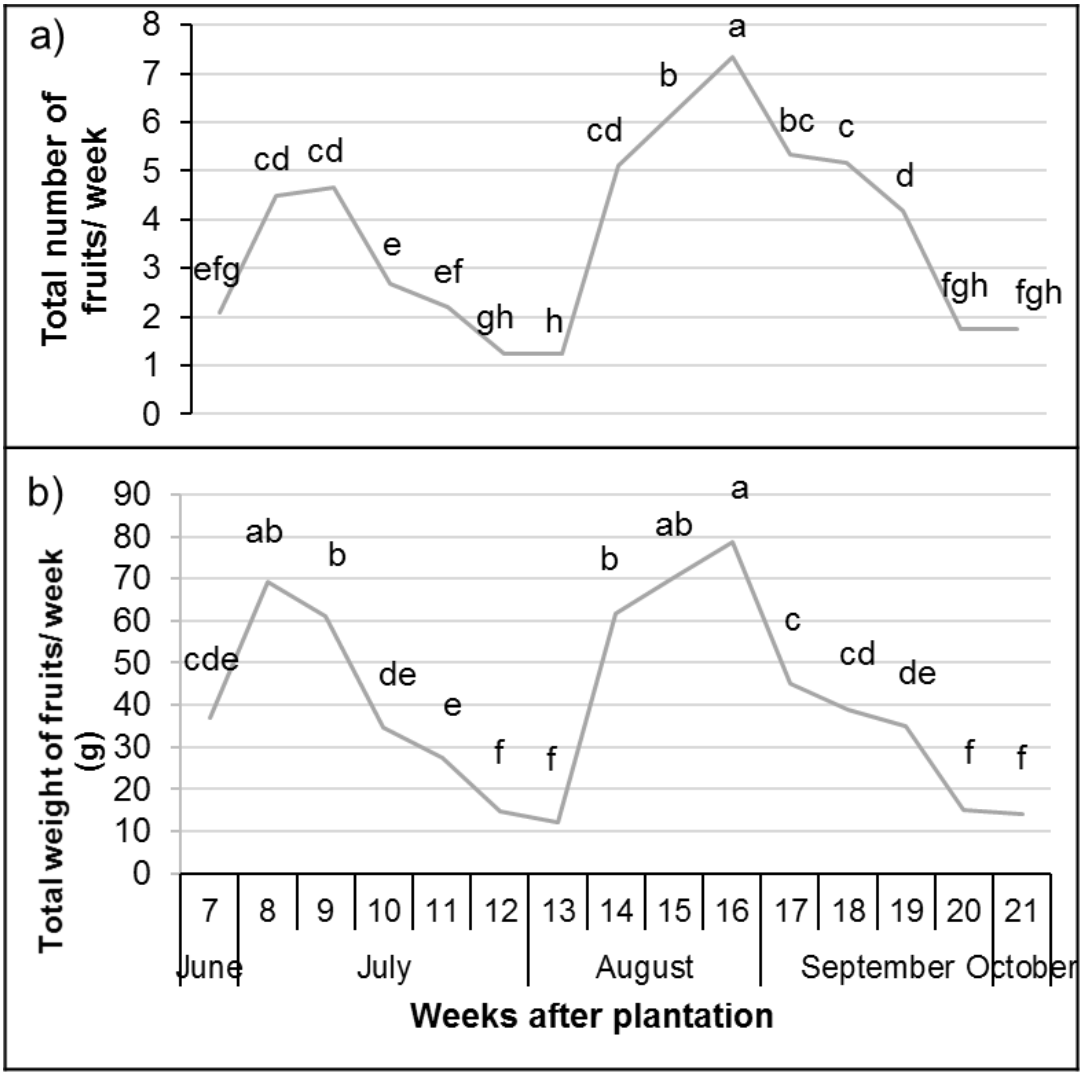


Annex B.26 Influence of time on the soluble sugar content (°Brix) of fruits from strawberry plants treated with biostimulants grown in (a) conventional and (b) organic growing systems. Means followed by the same letter are not significantly different (P≤ 0.05) (n=5).
*Treatments are different from their respective control

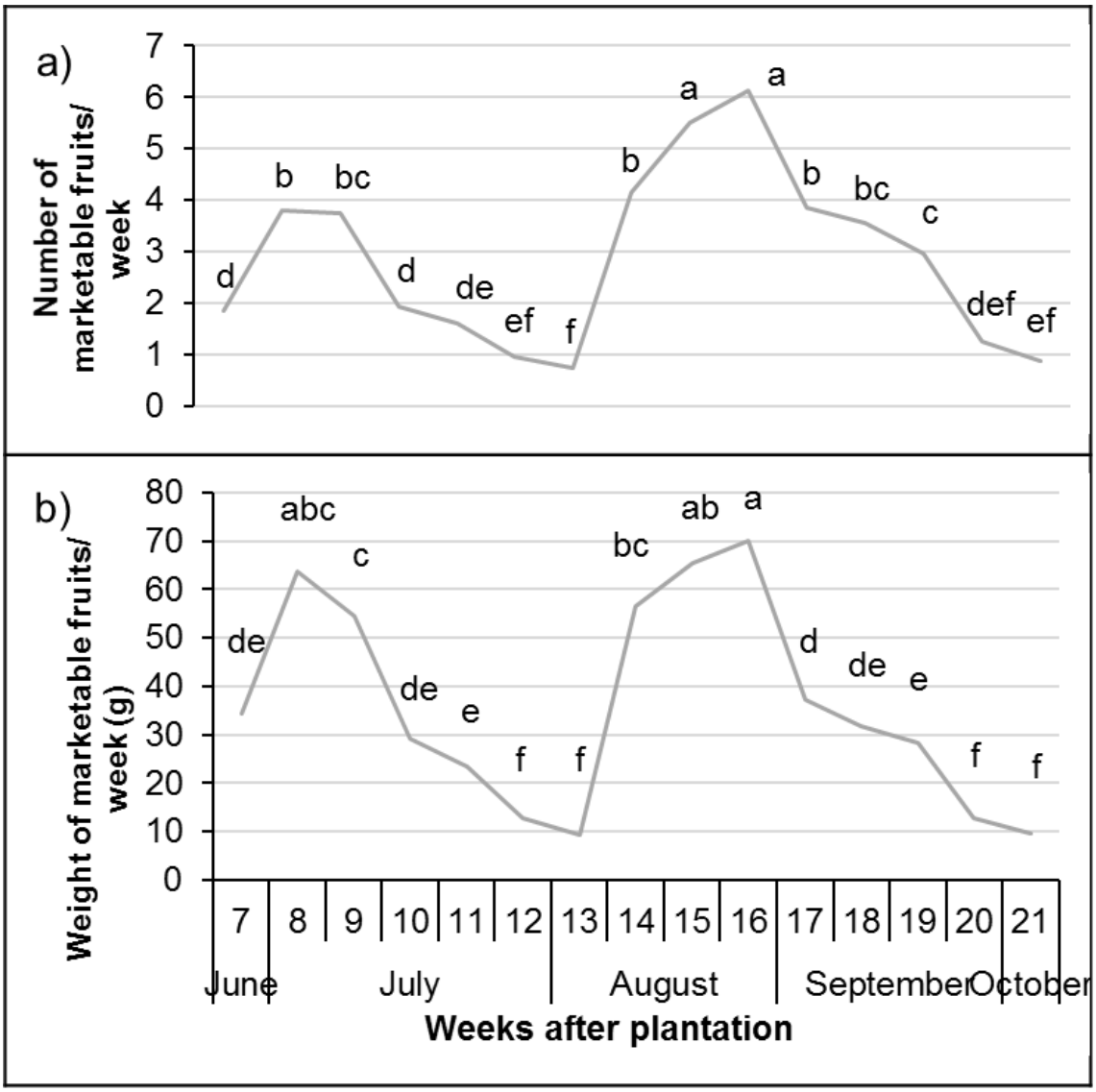
ANNEXE C



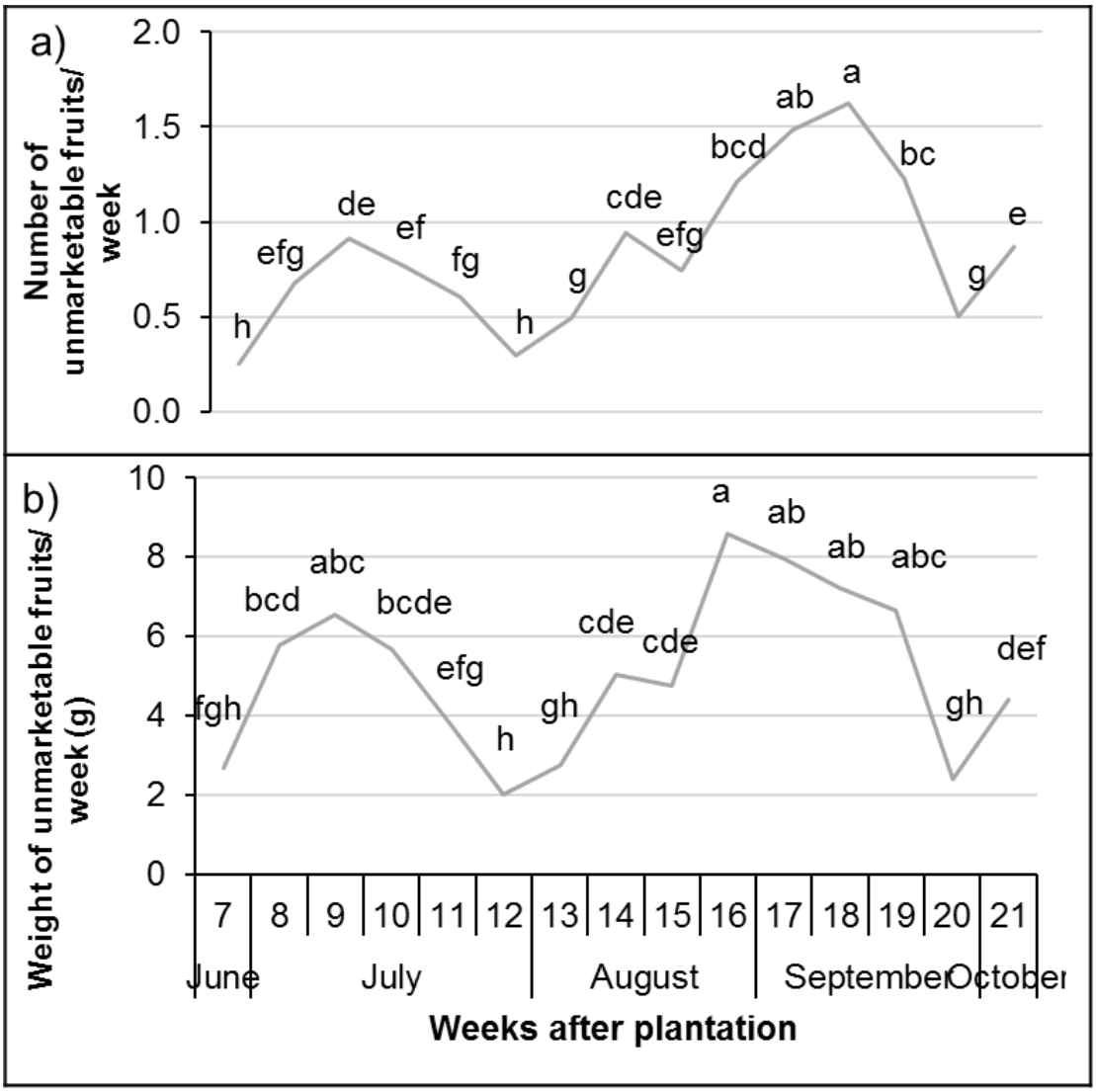
Annex C.1 Growth parameters variation of strawberry plants a) number of flowering stalks, b) number of crowns, c) diameter of stalks during the experimental period (summer 2018). Means followed by the same letter are not significantly different (P≤ 0.05) (n=72).



Annex C.2 Total yield (a) number, (b) weight variation of strawberry plants during the experimental period (winter 2018). Means followed by the same letter are not significantly different (P≤ 0.05).



Annex C.3 Marketable yield (a) number, (b) weight variation of strawberry plants during the experimental period (winter 2018). Means followed by the same letter are not significantly different (P≤ 0.05).



Annex C.4 Unmarketable yield (a) number, (b) weight variation of strawberry plants during the experimental period (winter 2018). Means followed by the same letter are not significantly different (P≤ 0.05).

ANNEXE D (OWC 2020 PAPER SUBMISSION-SCIENCE FORUM)

Organic World Congress 2020 | FRANCE

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AT THE COUVENT DES JACOBINS • RENNES MÉTROPOLE CONFERENCE CENTRE
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OWC 2020 Paper Submission - Science Forum

Topic 4 - Innovation in Organic farming: "thinking out of the Box"

OWC2020-SCI-849

IMPACT OF BIOSTIMULANTS ON GROWTH AND PRODUCTIVITY OF ORGANIC STRAWBERRIES

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[2]Institut sur la Nutrition et les Aliments Fonctionnels, Département de Phytologie, Université Laval, Québec City, Québec, Canada, G1V 0A6

Presentation Method: Oral or poster presentation

Full Paper Publication: Yes

Abstract: Organic strawberry production is faced with several biotic and abiotic stresses that compromise crop productivity and berry quality. In order to improve yield and berry quality, we have compared the potential beneficial effects of seven biostimulant treatments 1- control without biostimulant (CONTROL), 2- seaweed extract (SEAWEED), 3- mycorrhiza Rhizoglomus irregular (MYC), 4- mix of three bacteria, Azospirillum brasilense, Gluconacetobacter diazotrophicus, and Bacillus amyloliquefaciens (BACT), 5- combination of MYC+BACT, 6- MYC+BACT with a low fertilization (MYC+BACT/LF), and 7- citric acid-based (CITRIC) within a complete randomized block design with five replicates. Our results showed that some biostimulants did impact the soil relative abundance of fungi and

soil CO_2 efflux, while no effect was observed for the microbial activity (FDA) compared with the control. Leaf chlorophyll content and the chlorophyll fluorescence were not significantly affected by biostimulants. MYC decreased the number of flowering stalks (-18%) compared with control plants, while citric acid increased their dry root biomass (+35%). However, biostimulants did not affect the mineral content of leaves. Little effect of biostimulants on crop productivity was observed compared with control plants. However, MYC+BACT increased ºBrix (+11%), total polyphenols (+40%) and anthocyanins (+26%) of the berries compared with control. The use of a lower fertilization reduced plant growth and yield.

Keywords: bacteria, Brix, citric acid, fluorescein diacetate, Monterey, mycorrhiza

Introduction

Organic strawberry (*Fragaria x ananassa* Duch.) production often suffers from lower yield compared to conventional farming. This is generally related to a low nutrient availability for the crop and limited tools to control pest infestation[1]. On the other hand, biostimulants may help the plant to assimilate required nutrients and improve plant resilience to abiotic and biotic stresses[2]. This study aimed to investigate the effect of five plant biostimulants on the growth, development, productivity, and quality of organic strawberry plants grown in greenhouse.

Material and methods

The experiment was performed in a greenhouse complex located at Laval University, Quebec, Canada (Lat. 46°78ʾ N; long. 71°28ʾ W) from February 5th to July 11th, 2018. The treatments were 1- Control without biostimulant (CONTROL), 2- Seaweed extract (Acadian seaweed; soil application; SEAWEED), 3- Mycorrhiza (*Rhizoglomus irregular*; MYC), 4- *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus* and *Bacillus amyloliquefaciens* (BACT), 5- Mix of treatments 3 and 4 (MYC+BACT), 6- MYC+BACT with low fertilization (MYC+BACT/LF) and 7- Citric acid (Fungout®, AEF GLOBAL; CITRIC). The experiment defined as randomized complete block design with five replicates. Strawberries (cv Monterey) were grown in organic substrate (OM4 40 NF Wood with 40 % wood fibers + 50% peat + 10 % compost, Berger) and under natural light supplemented with HPS lamps

providing a PPFD of 162 µmol/m^2/s at the plant level, for a photoperiod of 16 hours (from 8 am to 24 pm), with CO_2 concentration between 600-700 µL L^{-1}, day/night temperature 18/13 ± 0.8 °C. Bumblebees as a natural pollinator (Biobest®, Ontario, Canada) were used to improve flower pollination inside the greenhouse. Plants were fertilized daily with liquid organic fertilizers (0.3% of Nature's Source (3-1-1), and 5.5 g of poultry manure pellets (Acti-sol 5-3-2) were applied to all treatments twice a month, except in treatment six.

Chlorophyll fluorescence (Fv/Fm maximum quantum efficiency of photosystem II and performance index) parameters, chlorophyll content (SPAD) and plant growth parameters (crown diameter and number of leaves, flowering stalks and crowns) were measured every month on three plants per experimental unit. Fruits were harvested once/ twice a week and classified in marketable and unmarketable fruits according to their shape and size. Soluble sugar content (°Brix) was evaluated monthly, while total polyphenols and anthocyanins were measured 3 times (July, August, September). Soil samples were collected to determine the soil microbial activity (FDA)[3]. At the end of the experiment, leaf area, fresh and dry biomass of the stems, leaves, and roots were measured on three plants per experimental unit. All data were analyzed by a two-way model of analysis of variance (ANOVA) using the MIXED procedure of SAS software (version 9.4, SAS Institute Inc. Cary, NC) with replicates as a random effect. Data were compared using LSD when effects were significant at a 5% confidence level ($P \leq 0.05$).

Results

Soil activity

Microbial activity (FDA) of the soil was not influenced by biostimulants, while low fertilization reduced its activity (Table 1). However, seaweed, MYC, BACT, MYC+BACT increased soil CO_2 efflux compared with control.

Physiological parameters

Our results showed that leaf Chlorophyll fluorescence (Fv/Fm and P Index) and Chlorophyll content (SPAD) were not influenced by biostimulants (Table 1). However, the low fertilization treatment (MYC+BACT/LF) induced lower values of P index and Chlorophyll content compared to the other treatments.

Plant growth

Table 1 and 2 showed that biostimulants had little impact on growth parameters, except for the number of flowering stalks that decreased by 14% for MYC compared with control, although the number of leaves (P=0.085) and of flowering stalks (P=0.082) tended to be higher than control for the citric acid treatment. Moreover, citric acid increased fresh (+32%) and dry (+35%) biomass of roots compared with control plants. The highest leaf area was observed in the SEAWEED treatment (10% higher than control; P=0.107), while shoot biomass of plants treated with citric acid tended to be higher than control plants (P=0.128, +18%). A low fertilization (MYC+BACT/LF) decreased the number of leaves and crowns, leaf area and shoot biomass compared to the control.

Yield and quality

Figure 1 showed that yield parameters were little influenced by the studied biostimulants compared with control, except for MYC+BACT (P<0.01) and citric acid (P=0.073) that increased the total number of fruits. A lower yield was observed for plants grown under low fertilization compared to the control. In terms of quality, MYC+BACT increased ºBrix (+11%), total polyphenols (+40%) and anthocyanins (+26%) of the berries compared with control (Table 2).

Discussion

Results of the present study showed that crop development and yield of organically grown strawberries were little affected by the studied biostimulants. However, although not significant at P<0.05, foliar citric acid application tended to increase yield of berries. On the other hand, the use of a mixture of mycorrhiza and bacteria (MYC+BACT) increased the ºBrix, polyphenols and anthocyanins of the berries compared to the control and the use of mycorrhiza or the bacteria alone, while citric acid tended to increase the anthocyanin content (*P*=0.068). Our results agree with study showing the positive effects of inoculation with plant growth prompting bacteria[4] on the sugar and anthocyanin concentration of strawberry plants cv. Elyana. Besides, we observed that citric acid and seaweed extract had the capacity to increase root biomass and leaf area, respectively. Similar results were reported in roses [5, 6].

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Table 1. Effect of studied biostimulants on physiological parameters (Fv/Fm, P Index, and chlorophyll content (SPAD)), and growth parameters (Number of leaves, number of flowering stalks, number of crowns, and diameter of crowns).

Treatments	Fv/Fm	P Index	SPAD	Number of leaves	Number of flowering stalks	Number of crowns	Diameter of crowns (mm)
CONTROL[z]	0.805	2.952a	38.1a	15.40ab	4.69abc	3.38ab	40.34
SEAWEED	0.808	3.104a	37.3a	13.92bc	4.45bcd	3.20abc	33.62
MYC	0.805	2.801a	38.2a	15.02b	4.02d	3.15bc	38.03
BACT	0.803	2.840a	37.7a	15.11b	4.90ab	3.39ab	36.96
MYC+BACT	0.804	2.960a	37.2a	15.33ab	4.87abc	3.35ab	36.74
MYC+BACT /LF	0.801	2.348b	34.4b	12.82c	4.26cd	2.88c	29.72
CITRIC	0.808	2.981a	38.1a	17.01a	5.25a	3.58a	39.34
Biostimulant	ns	***	***	***	*	*	ns

[z]CONTROL= without biostimulant; SEAWEED = seaweed extract; MYC = mycorrhiza *Rhizoglomus irregulare*; BACT = three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT = combination of treatments MYC and BACT; MYC+BACT/LF = combination of treatments MYC and BACT with low fertilization; CITRIC = citric acid-based formulation (Fungout® AEF GLOBAL Inc., foliar spray with pH=6.2).

[y]***, Significantly different at P<0.001; **, significantly different at P<0.01; *, significantly different at P<0.05; ns, not different at P>0.05.

[x]means with different letters are significantly different at P<0.05.

Table 2. Effect of studied biostimulants on fruit quality (Brix, total polyphenol, and anthocyanins), microbial activity (FDA), leaf area, shoot and root fresh and dry biomass.

Treatments	Brix	Total polyphenols (mg GAE /100gDW)	Anthocyanins (mg/100g DW)	FDA (µg/h/g dry soil)	Leaf Area (cm^2 $plant^{-1}$)	Shoot fresh biomass	Shoot dry biomass	Root fresh biomass	Root dry biomass
CONTROL[z]	8.88b	6184b	241bc	885.0a	2347.5ab	125.44ab	29.7a	46.2bc	6.72bc
SEAWEED	9.25ab	7018b	256b	881.8a	2591.3a	136.66ab	29.54a	47.0bc	6.62bc
MYC	8.80b	7101b	231bc	906.8a	2179.8ab	116.34b	26.93a	43.1bc	6.39bc
BACT	8.95b	7202b	228bc	758.6ab	2343.7ab	120.65ab	27.93a	42.3bc	5.99bc
MYC+BACT	9.88a	8633a	302a	898.55a	2055.8b	111.1b	26.13a	49.9ab	6.98ab
MYC+BACT/LF	8.83b	6841b	221c	555.85b	1172.5c	62.902c	14.99b	38.1c	4.98c
CITRIC	9.44ab	6772b	275b	830.04a	2492.7ab	147.83a	32.45a	60.99a	9.04a
Biostimulant	*	*	*	*	***	***	***	***	***

[z]CONTROL= without biostimulant; SEAWEED = seaweed extract; MYC = mycorrhiza *Rhizoglomus irregulare*; BACT = three bacteria *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Bacillus amyloliquefaciens*; MYC+BACT = combination of treatments MYC and BACT; MYC+BACT/LF = combination of treatments MYC and BACT with low fertilization; CITRIC = citric acid-based formulation (Fungout® AEF GLOBAL Inc., foliar spray with pH=6.2).

[y]***, Significantly different at P<0.001; **, significantly different at P<0.01; *, significantly different at P<0.05; ns, not different at P>0.05.

[x]means with different letters are significantly different at P<0.05.

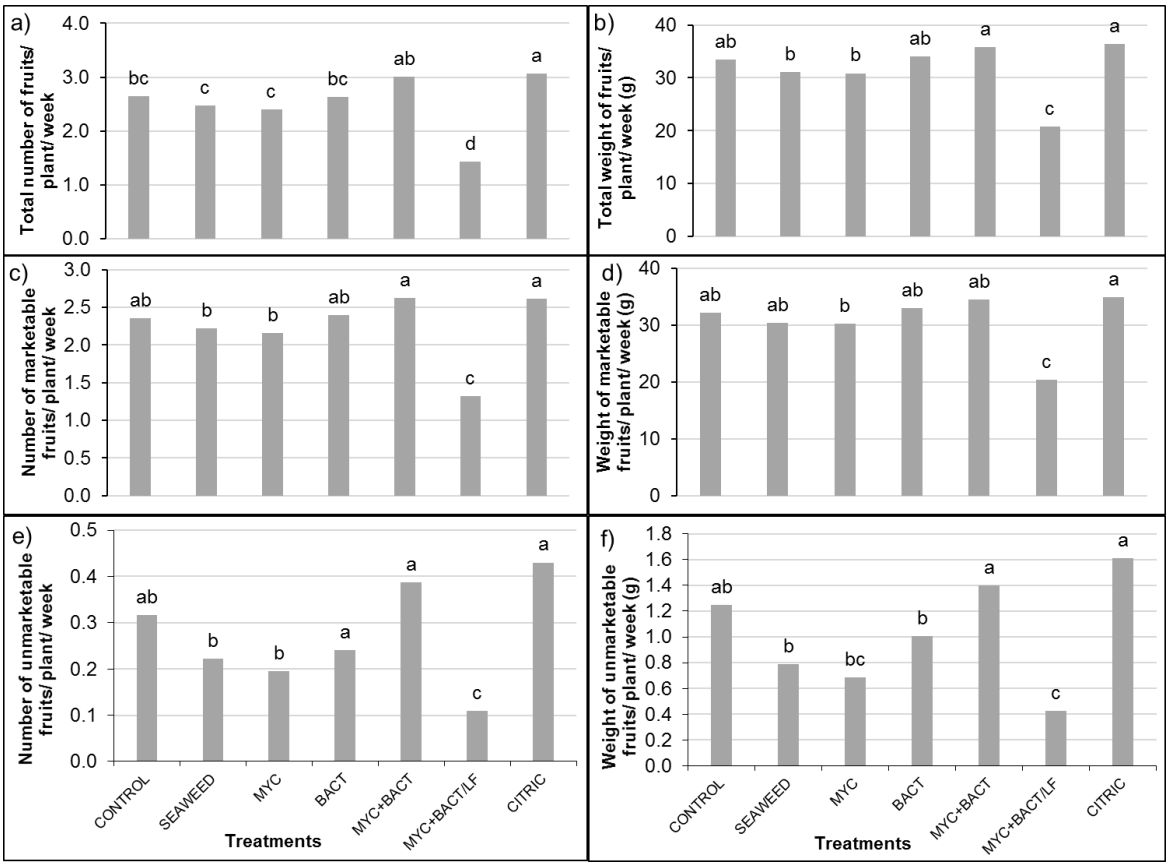

Figure 1. Effect of studied biostimulants on total number and weight of fruits/ plant/ week (a, b), number and weight of marketable fruits/ plant/ week (c, d) as well as number and weight of unmarketable fruits/ plant/ week (e, f) during experiment, winter 2018. Different letters indicate a significant difference ($P<0.05$) among treatments by LSD test (n=55).